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CANADIAN THESES ON MICROFICHE | THÈSES CANADIENNES SUR MICROFICHE

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TITLE OF THESIS/*TITRE DE LA THÈSE* Biosystematic Studies in the Genus Hirschioporus

UNIVERSITY/*UNIVERSITÉ* Univ. of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED/ *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* PhD.

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE DEGRÉ* 1974

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THE UNIVERSITY OF ALBERTA

BIOSYSTEMATIC STUDIES IN THE GENUS, *HIRSCHIOPORUS*

by

© JAMES ALVIN TRAQUAIR

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

MYCOLOGY

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

FALL, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "BIOSYSTEMATIC STUDIES IN THE GENUS, *HIRSCHIOPORUS*" submitted by JAMES ALVIN TRAQUAIR in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Mycology.

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ABSTRACT

Hirschioporus pargamenus (Fr) Bond. & Sing., *H. abietinus* (Dicks. ex Fr.) Donk and *H. subchartaceus* (Murr.) Bond. & Sing. are similar polypores with characteristic violaceous pore surfaces and are frequently the cause of white-rot in trees of Western Canada. Variation in macroscopic basidiocarp features such as shape, size and pubescence has made species delineation based on traditional taxonomic methods very difficult.

Configuration of the hymenial surface and texture of the context are practical macroscopic distinctions by which the species can be identified and are supported by differences seen in the surface mats, growth rates of the vegetative mycelium and structure of basidiocarps produced under standard culture conditions. Developmental studies are essential for the recognition of thick- and thin-walled generative hyphae and skeletal cells which form the structural basis for macroscopic features but the vegetative mycelium and basidiocarps of the three species are constructed of the same three hyphal types. A general pattern of species intersterility and species intrafertility is observed in paired monokaryotic isolates and in paired monokaryotic and dikaryotic isolates by using the production of clamp connections as a criterion. The interaction of dikaryotic mycelia of different *Hirschioporus* species with each other and with dikaryotic mycelium of unrelated polypores is expressed in the formation of demarcation lines, clasping branches and coloured zones in culture. Such interactions are interpreted as having taxonomic significance for these species.

Whether results are obtained from macro-morphological, micro-morphological, developmental, cultural or genetical studies, they are inadequate by themselves as a base for taxonomic decisions about species in the genus *Hirschioporus*. It is necessary to correlate information from all of these sources in order to delineate the taxa with confidence. At the same time, the many similarities between the species in terms of colour of the pore surface and microstructure of vegetative mycelium and basidiocarps provides evidence for a very close relationship between species in this genus.

ACKNOWLEDGEMENTS

I express my sincere gratitude to Dr. L. L. Kennedy for her encouragement during this study and for her patient supervision during the preparation of the thesis.

Many thanks are extended to Bill Wong and Gordon McPherson for their assistance in the collection of fresh material and to Dr. J. Parmelee at the Biosystematics Research Institute, Ottawa and Prof. J. L. Lowe at the New York State College of Forestry, Syracuse University, Syracuse, N.Y. for the loan of herbarium material.

I acknowledge financial support provided by a Graduate Research Assistantship and Graduate Teaching Assistantship from the Department of Botany and two Post-graduate Scholarships from the National Research Council of Canada. This work was also supported by a grant to Dr. Kennedy from the National Research Council.

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"Our simple problems often grew
To mysteries, we fumbled over,
Because of lines we nimbly drew
And later neatly stumbled over"

Piet Hein

(Munk, 1962)

CHAPTER I

INTRODUCTION

The polypores, commonly termed "bracket-fungi" belong to a large, heterogeneous family of higher basidiomycetes, the Polyporaceae which constitutes the greater proportion of wood-decay fungi. A few species are parasitic causing serious losses through decay of living trees, but most polypores are saprobes responsible for losses due to decay of timber and wood-products. Although the destructive activities of these organisms have been emphasized, it must be pointed out that their ecological role as decomposers of wood debris is of primary importance in the cycling of elements in the forest ecosystem. However, it is the economic implications of decay and its prevention that have stimulated intensive studies of the biology of polypores in nature and in culture. Characteristics of the generally tough and easily preserved fruiting-bodies remain the traditional means by which these fungi are identified. Cultural and genetical studies have been reported for a small number of species and these have provided fundamental information towards an understanding of their growth and identification. As yet, the taxonomy of the polypores is in a state of confusion, and cultural studies elucidating the growth and development of many species have still to be done.

The three polypores used in this study, *Hirschioporus abietinus* (Dicks. *ex* Fr.) Donk, *H. pargamenus* (Fr.) Bond. & Sing. and *H. subchartaceus* (Murr.) Bond. & Sing. constitute a closely related group whose

basidiocarps have characteristic violaceous hymenial surfaces containing capitate, crystal-incrusted cystidia. Common in Western Canada, these fungi cause a white, pocket-rot in the sapwood of coniferous and deciduous trees. On account of the similarities between these species, their identification has presented difficulties, compounded by problems of variation in basidiocarps with age, position on the log and habitat in which they are produced. Consequently, the work of early mycologists (Overholts, 1915a; Lloyd, 1917; Rhoads, 1918) indicates broad concepts of these species. Any variants have been considered as forms of the "typical" basidiocarps. For example, Rhoads (1918) distinguished between the basidiocarps of *Polyporus* (*Hirschioporus*) *pargamenus* and *P. abietinus*, but considered *Coriolus* (*Hirschioporus*) *subchartaceus* to be a thicker, poroid form of *P. pargamenus* whose basidiocarps develop thin, lacerate, tooth-like tubes with age. Confusion at the species level is aggravated by the unsettled state of the classification of the family. A brief survey of the taxonomy will show that these three species have been classified in several genera, the most common being *Polyporus*, *Polystictus*, *Coriolus*, and *Hirschioporus*.

The development of the various systems used to classify the Polyporaceae is related to the knowledge of the taxa and, in turn, to the concepts and approaches taken by the taxonomist at the time. The initial works of Fries (1821, 1828, 1836-1838) have become the foundation for all subsequent systematic studies of the polypores. His exclusive use of macroscopic features, which has become the traditional approach, resulted in the broad generic concepts characteristic of the early conservative taxonomists. According to shape, attachment, texture and pubescence of their basidiocarps, *Polyporus abietinus* Dicks. *ex* Fries (Fries, 1821) and

P. pargamenus Fries (1838) were described in a broad genus containing many unrelated species, but were categorized in the sub-group, "Coriacei" whose members possessed sessile, fibrous-pubescent, leathery (coriaceous) basidiocarps. In 1851, Fries transferred these taxa to the genus *Polystictus* which he erected to include those species of *Polyporus* whose pores matured toward the base of the basidiocarp, the "substance of the pores being vertically opposed to that of the pileus" (Ames, 1913). Difficulties in recognizing this genus which was still large and heterogeneous resulted in a return to *Polyporus* (Fries, 1874), a decision supported by most subsequent mycologists.

Early successors to Fries sought smaller groupings of closely related taxa and provided an innovation to the traditional approach by using microscopic basidiocarp features, primarily those of the hymenium (spores, basidia, cystidia). The microscopic approach to identification and classification improved the descriptions of species and genera, but there was a certain reluctance to depart from Friesian concepts. Consequently, the new genera created by the "splitters" were largely subdivisions of *Polyporus* raised to generic rank. For example, Fries' subgroup, "Coriacei" is not markedly different from the genus, *Coriolus* erected by Quélet and which included *C. pargamenus* (Fr.) Pat., *C. abietinus* (Dicks. *ex* Fries) Quélet and *C. subchartaceus* Murr. (Quélet, 1888; Patouillard, 1900; Murrill, 1907). A comprehensive treatment of the polypores of France by Bourdot and Galzin (1928) deals with the first two of the above taxa but not *C. subchartaceus*.

The use of microscopic features gave polypore taxonomists increased confidence in the delineation of species and genera. More genera were split off with the result that since the time of Fries (1821) the number

of polypore genera has increased from two to three hundred. Cooke (1959) considered approximately one hundred of these genera to be valid. Based on careful studies of the polypores of the Netherlands, Donk (1933) erected the genus, *Hirschioporus*, indicating *H. abietinus* as the type species because of its violaceous pore surface bearing crystal incrusted cystidia and its resinous, brittle context when dried. Bondartsev and Singer (1941) added *C. subchartaceus* and *C. pargamenus* to this group, although Donk considered the fibrous-coriaceous context of *C. pargamenus* to be distinct enough to keep that species in *Coriolus*. Bondartsev's monumental treatise (1953) represents careful study and an incorporation of many observations of microscopic features, not only of the hymenium, but of hyphae that make up the rest of the basidiocarp. He recognized the close relationship between *H. pargamenus* and *H. abietinus* in terms of microscopic and macroscopic characters of the basidiocarp but did not describe those of *H. subchartaceus*. Overholts (1915b) grouped the three taxa in the genus, *Polyporus*, in his early study, *The Polyporaceae of the middle-western United States*. His treatment of the polypores in 1953 is detailed but reflects a preference for the conservative system as being more practical for purposes of identification. At the same time, he recognized species affinities within the large genus, *Polyporus*, and grouped the three taxa studied here close together on the basis of their similar macroscopic and microscopic basidiocarp features.

As microscopic analysis of basidiocarp anatomy was receiving the increasing attention of twentieth century mycologists, the hyphal structure of polypores was emphasized. Following the studies of Corner (1932a,b) who carefully examined the hyphae and described the basidiocarp development of *Polystictus xanthopus* and *Fomes levigatus*, Cunningham (1954,

1965) produced a system of classification of the Polyporaceae using hyphal characteristics as diagnostic features. In his classification, he described *Trichaptum* (*Hirschioporus*) *pergamenum* (Fr.) G.H. Cunn. and placed this species in a small genus originally proposed by Murrill with the type, *Polyporus trichomallus* Berk. & Mont. which Overholts (1953) recognized as having similarities to the species reported upon in this thesis. In his classification, Cunningham still used macroscopic characters as key features. Moreover, his rather premature use of hyphal characteristics of the basidiocarps has resulted in a classification that is just as artificial as the others (Teixeira, 1962).

As the search for new identifying characters continued, a different taxonomic approach developed out of the studies of the vegetative mycelium of polypores in culture. The work of Nobles (1948, 1965) has shown that cultural characters are identifying features. She has constructed a scheme for the classification of polypores using such characteristics (1958b, 1971). But in her papers, she emphasizes the need for correlative studies of basidiocarps in nature. Her studies of *H. pargamenus* and *H. abietinus* in culture indicate a very close relationship between these taxa, but no mention is made of *H. subchartaceus*.

Cultural studies of polypores have focused attention on the use of genetical studies in the separation of "biological" species. Among Canadian mycologists, Mounce, Macrae and Nobles are primarily responsible for advocating the use of the "clamp criterion" for the conspecificity of taxa. Macrae (1941) has used this test in her studies of *Polyporus pargamenus* and *P. abietinus* and in her studies of *Hirschioporus* (*Polyporus*) *abietinus*, *H. fusco-violaceus*, and *H. laricinus* (1967).

More recently, chromatographic and electrophoretic techniques used

in the study of enzymes and secondary metabolites have been the basis for a chemotaxonomic approach to the study of polypores. Enzyme studies by Shannon *et al.* (1973), however, using starch-gel electrophoresis indicate that *P. pargamenus* and *P. abietinus* grown in culture have fewer bands in common with each other than with species which appear unrelated using basidiocarp features. Variability of isolates, the effects of age and cultural conditions compound the methodological problems and impracticalities of using these techniques for standard identification of species of polypores (Tyler, 1971), and they have not been used in the study reported here.

Variation in basidiocarp structure and the occurrence of intermediate and overlapping forms have resulted in the unclear delineation of species and have presented major obstacles in the use of existing classifications which employ morphological characters of the basidiocarps in the identification of polypores. To attain a natural classification into genera whose species share many similar characteristics, we must accurately circumscribe the species and then use an agglomerative procedure in the clustering of related species into genera. Each approach to the problem of identification, whether it is macro-morphological, micro-morphological, cultural or genetical will lead to a different interpretation of species and genera if considered by itself. Biosystematic studies as used by vascular plant taxonomists are equivalent to experimental taxonomy (Lawrence, 1951) and are entirely applicable to studies of polypores. The biosystematist seeks an "overall picture of biological relationship". He attempts to correlate evidence from morphology with cytology and genetics, and cultivates the taxa in uniform and varied environments as an aid to the delimitation of species and

determination of generic relationships. Because *Hirschioporus* species can be readily cultivated in the lab and produce basidiocarps in this environment, biosystematic studies are advocated in the examination of species whose basidiocarps are highly variable in the field. Developmental morphology is not customarily stressed in biosystematic studies of vascular plants but, I find that the developmental approach is fundamental to a correct interpretation of micro- and macro-morphology in the field and in culture. Biosystematic studies of the complete life-cycle are lacking for many polypores. The aim of the study is to obtain a more precise concept of each species and of the genus *Hirschioporus* by interrelating the findings from several approaches.

Macrostructure of basidiocarps as the first and traditional source of characters, is presented in Chapter II. Modern workers tend to underestimate the value of such characters because of the variation encountered in them. With respect to *Hirschioporus* species, basidiocarp macrostructure has proven to be a useful source of characters if considered along with the results of other approaches. Studies of basidiocarp microstructure reported in Chapter III, evolved out of the traditional approach and culminated in detailed investigations of hyphal anatomy and basidiocarp development. Developmental studies are much needed for these taxa. Similarities between *Hirschioporus* species with regard to microstructure of the basidiocarp prompted a study of the vegetative mycelium and basidiocarps produced in culture (Chapter IV). Cultural studies have become standard procedure in the identification of polypores, but they involve basidiocarps produced under these conditions. The fortuitous production of cultural basidiocarps by the three *Hirschioporus* species enabled a more complete

examination of the life-cycle in a standard environment. Cultural studies led into the use of genetical tests to differentiate between these species (Chapter V). Intersterility of *Polyporus* (*Hirschioporus*) *abietinus* and the other two taxa has been reported by Macrae (1941), but she did not provide a conclusive distinction between *P. pargamenus* and *P. subchartaceus*. Considering their macro-morphological and micro-morphological similarities in the basidiocarps and vegetative mycelium in nature and in culture, further genetical studies of these two species are warranted. The new approach proposed in Chapter VI developed from cultural and genetical studies in which mycelial interactions were noted between *Hirschioporus* species. In this chapter, macroscopic reactions in the form of coloured interaction zones and microscopic reactions in the form of specialized branches on the hyphae in these zones are reported. The different reactions by these taxa when confronted by unrelated polypore species growing with them in culture is thought to have taxonomic significance.

Observations are summarized in Chapter VII, and each approach to the delimitation of species and the elucidation of relationships in the genus, *Hirschioporus*, is evaluated in terms of the results obtained by a consideration of the "sum total of characters" (Munk, 1962).

CHAPTER II

BASIDIOCARP MACROSTRUCTURE

Introduction

The macrostructural approach to the taxonomy of the polypores has involved a consideration of the distribution and substrates decayed by them as well as a description of basidiocarp morphology. Such an approach is taken in this chapter to illustrate distinctions and similarities between three poroid species of the genus, *Hirschioporus*.

The identification of these fungi has resulted in some confusion because of generalizations concerning the substrate preferences apparent to collectors. There has been a tendency to rely upon the general observation that *H. pargamenus* occurs on hardwoods while *H. abietinus* occurs on softwoods. However, exceptions to this "rule of thumb" have been reported (Rhoads, 1918; Overholts, 1953; Macrae, 1941, 1967). For example, Rhoads and Overholts listed *Pinus* and *Tsuga* as substrates for *H.* (*Polyporus*) *pargamenus*, while Macrae reported the occurrence of *H. abietinus* on the wood of *Betula papyrifera*. The work of Murrill (1907) illustrates how firmly this apparent substrate preference was instilled in the minds of early mycologists. He disagreed with Fries' (1838) application of the name, *P. pargamenus*, to material collected on *Pinus* in Arctic North America. Consequently, he listed this name as a synonym for *Coriolus* (*Hirschioporus*) *abietinus* and gave the taxa known as *H. pargamenus* the name, *C. prolificans* (Fries) Murr., now accepted as a synonym for *H. pargamenus* (Overholts, 1953; Bondartsev, 1953). At this time, Murrill

also described a new species *C. subchartaceus* from specimens collected on *Populus tremuloides* in Colorado. Rhoads (1918) and Overholts (1953) have reported this thick, poroid species on *Populus* in Western United States and Canada. Personal collections of the three species have been made in various localities, particularly in Western Canada, in order to test the hypothesis that there is a substrate preference.

The original descriptions of these taxa are based on macrostructure of basidiocarps. Macroscopic characters include form and size of the basidiocarp, colour, pubescence, configuration of the hymenial surface, and texture of the context. Murrill's work which reflects the use of such basidiocarp features, is the first comparision of all three taxa. Overholts' (1953) keys, which are widely used in the identification of polypores in North America, distinguish *H.* (*Polyporus*) *pargamenus* from *H. abietinus* on the basis of colour and pubescence of the upper surface of the basidiocarp. *H. pargamenus* and *H. subchartaceus* are separated by means of context thickness and configuration of the hymenial surface. Similarly, in 1953, Bondartsev has reported macroscopic distinctions between basidiocarps of European collections of *H. pargamenus* and *H. abietinus*, but has not reported on *H. subchartaceus*.

It is unfortunate that these macroscopic characters of basidiocarps are subject to variation depending upon the environment in which they are produced and the state of maturity at the time they are collected. Hence, early descriptions are somewhat ambiguous in delimiting *Hirschioporus* species. The occurrence of intermediate forms has resulted in the failure by many mycologists to recognize the distinction of *H. subchartaceus* from *H. pargamenus*. In this chapter, macrostructure of the basidiocarps of the three species collected in a number of habitats and at various

stages of maturity are described. Comparative observations of fresh collections and herbarium material are summarized in technical descriptions.

Materials and Methods

Fresh collections from various substrates throughout Alberta and parts of British Columbia and Saskatchewan were examined. Additional fresh basidiocarps were obtained from the North West Territories, Minnesota, Iowa and Michigan. Dried material was examined from herbaria at the University of Alberta, Edmonton (ALTA) and the Biosystematics Research Institute, Ottawa (DAOM) as well as from the personal herbarium of Prof. J.L. Lowe at the New York State College of Forestry, Syracuse. Symbols for herbaria are taken from the *Index Herbariorum, The Herbaria of the World*, 5th ed. (Lanjouw and Stafleu, 1964). A complete list of the collections is presented in Appendix I and II.

In previous descriptions it is unclear as to where measurements of basidiocarp size have been taken. The measures of width and length given here were made at the widest and longest part of the basidiocarp; thickness was measured at one-half the maximum distance from base to margin.

Observations

Distribution and substrates

The three *Hirschioporus* species are found in close proximity in geographic locations characterized by mixed, deciduous and coniferous forest (Fig. 1). In Western Canada, basidiocarps of *H. subchartaceus* and *H. abietinus* are collected more frequently than those of *H. pargamenus*.

The distribution of these taxa is related to the location of woody substrates. Decay by *H. subchartaceus* and *H. pargamenus* is restricted to hardwoods, while that of *H. abietinus* is confined to the wood of coniferous trees (Table 1). *H. pargamenus* is found on *Betula*, occasionally on logs in close proximity to *Populus* decayed by *H. subchartaceus*. In the aspen stands of parkland areas, *H. subchartaceus* is frequently collected on fallen and standing dead poplar (Fig. 1). *H. abietinus* decays various conifers, the most common being *Picea* and *Pinus*. Even where spruce and poplar logs are adjacent, *H. abietinus* is collected on *Picea* while *H. subchartaceus* is collected on the *Populus* only. Among the collections observed from Eastern North America, where a greater variety of substrates are available, *H. subchartaceus* is found decaying wood of other species such as *Betula* and *Prunus*. Also, *H. pargamenus* is reported on *Quercus*, *Prunus*, *Fagus*, *Acer* and *Vitis* as well as *Betula* (Table 1).

These fungi share similar habitats such as slash piles, old bogs, burned over forest and edges of wooded regions. *H. pargamenus* is found in moist, shaded localities but never in the warmer, more exposed sites in which the other two species can be collected. Moisture is critical to their development, and consequently, basidiocarps are abundant where more humid conditions prevail on the lower surface of raised logs and the shaded side of logs and stumps. Fruiting-bodies are seldom found on de-barked logs, and furthermore, they are not collected on fallen trees in exposed, wind-swept mountain slopes.

Basidiocarp size and shape

Sizes overlap depending upon the age of the basidiocarp and the environment in which it is produced. Basidiocarps of these three species

are mature and sporulating when 2-3 mm wide. Therefore, measurements of the smallest basidiocarps are not distinguishing features. Small, thick basidiocarps are found in exposed sites while large, thin basidiocarps are characteristic of moist, shaded localities. In terms of length, width and thickness respectively, single basidiocarps of *H. pargamenus* are 0-6 x 1-9 x 0.1-0.4 cm. *H. abietinus* basidiocarps are 0-6 x 1-3 x 0.1-0.2 cm, while those of *H. subchartaceus* are 0-6 x 0.5-6 x 0.1-0.9 cm.

H. pargamenus, *H. abietinus* and *H. subchartaceus* are similar in basidiocarp shape and form. Depending upon their position on the decaying log, basidiocarps may be sessile, narrowly attached (wedge-shaped) or broadly attached (dimidiate), solitary or laterally confluent, imbricate (overlapping), effused-reflexed or resupinate. The basidiocarps are annual or biennial in which renewed growth over the old basidiocarps surface is observed. This re-growth is particularly noticeable where basidiocarps have been reorientated with respect to gravity on disturbed logs (Fig. 15).

Sessile basidiocarps of *H. pargamenus* are usually attached by a narrow base (Fig. 6) to the side of the log, and on the top of the log, they assume a circular or funnel-like shape (Fig. 5). Imbricate (Fig. 3) basidiocarps are common, occurring over the log or stump surface. On the lower side, basidiocarps are resupinate or apileate (Fig. 5). However, these resupinate basidiocarps are unlike those described for *Poria* species because they are not attached firmly to the substrate but develop from a central point of attachment. When the basidiocarp reaches the edge of the log by radial growth, part of the margin becomes reflexed to form an effused-reflexed basidiocarp (Figs. 2, 7). Sessile, imbricate, effused-reflexed and resupinate (Figs. 8-13) basidiocarps are observed for

H. abietinus. The sessile, single basidiocarps are usually broadly attached and slightly decurrent at the base giving the pileus a "hood-like" appearance (Bondartsev, 1953). The basidiocarps of *H. subchartaceus* are also broadly attached (Fig. 17) although narrowly attached basidiocarps have been observed (Fig. 19). The entire log surface may be covered with solitary or imbricate basidiocarps which are often laterally confluent (Figs. 14, 18). On the sides and undersurface of logs, effused-reflexed and resupinate basidiocarps (Figs. 16, 20) are observed.

The upper surface of the basidiocarp

Colour and pubescence of the upper surface provide characters by which the three taxa can be distinguished generally, although the environment in which the basidiocarps develop and their age affect the expression of these characteristics, particularly colour. The surface of basidiocarps of *H. pargamenus* is white to tan in colour with concentric darker brown zonations (Fig. 22) which are distinct from the blackish, glabrous bands marking the initiation of renewed growth (Fig. 23). The surface is usually "velvety-pubescent" (Overholts, 1953) with concentric sulcations (Figs. 21, 22) and occasionally silky to nearly glabrous with appressed fibrils radiating from the base of the basidiocarp. *H. abietinus* basidiocarps are white to gray with age on the upper surface (Fig. 25). Concentric black zonations (Figs. 24, 26) are likewise observed in those basidiocarps showing renewed growth. The basidiocarp surface is conspicuously "strigose" (Overholts, 1953) and sulcate although this character is less obvious in young basidiocarps produced in moist conditions. In vertical sections of basidiocarps, it is observed that the pubescence contributes to most of the basidiocarp thickness (Fig. 31).

H. subchartaceus basidiocarps are difficult to distinguish from those of *H. abietinus* on the basis of characteristics of the upper surface. Usually, the surface of the former is white to grey, "villose-tomentose" (Overholts, 1953), inconspicuously zonate and sulcate (Figs. 27, 28). Blackish, glabrous zones are observed on the surface of older basidiocarps exposed to changing environmental conditions of temperature and moisture (Fig. 29). If suddenly dried, the growing margin of the basidiocarps of this and the other species becomes blackish and brittle and when the basidiocarp resumes growth, the black area becomes the dark zone on the basidiocarp surface.

The context and tubes of the basidiocarp

Thickness, texture and colour of the context and tube layer vary for these three species. The context of the basidiocarps of *H. pargamenus* is 1-4 mm thick, white to creamy in colour and "fibrous-coriaceous" (Bondartsev, 1953) in texture (Fig. 30). The context of *H. abietinus*, on the other hand, is 1 mm thick or less, creamy to reddish-brown upon drying and coriaceous (leathery) to cartilaginous (brittle) when dried (Fig. 31). It appears as a distinct, dark, narrow line in vertical sections of the basidiocarp. *H. subchartaceus* basidiocarps generally have a thicker context (Fig. 32), 2-5 (-10) mm which is creamy-white in colour and fibrous-coriaceous in texture. In fact, longitudinal sections of basidiocarps are commonly triangular in outline due to the thickened base.

The tube layer of all three taxa is creamy to light brown in colour when sectioned vertically. It is leathery in texture when young but dries rigid. Tube lengths vary from 1-3 mm but tend to be longer in the basidiocarps of *H. pargamenus*.

The lower surface of the basidiocarp

The pore surface of all three species is violaceous in colour (Figs. 33-35), the intensity of which varies with the age of the basidiocarp and its exposure to light during development. Young fruiting-bodies of *H. pargamenus* have a dark, violet pore surface fading to a brownish colour with age. When produced in a shaded environment, the lower surface is pale, pinkish to white in some basidiocarps. The pigmentation occurs in the upper and lower surface of the growing basidiocarp margin and the mouths of tubes. Pinkish to violaceous patches have also been observed on the surface of disturbed basidiocarps where growth has resumed. Similar observations are made for the degree of pigmentation and its localization in the basidiocarps of *H. abietinus* and *H. subchartaceus*.

Configuration of the pore surface is an important feature used in identifying these species in the field. Variation exists with the age of the basidiocarp and careful note must be taken of the position of the basidiocarps on the log in interpreting the apparent intermediate conditions in the configuration of the hymenial surface of any of the taxa. Tubes formed behind the thin (less than one millimetre), narrow (0.2-0.6 mm) growing margin of the basidiocarps of *H. pargamenus* are seriately placed (Fig. 37). Their mouths are round to angular and soon become conspicuously tooth-like (irpiciform) due to the irregular extension of the thin (30-50μ) tube walls (Fig. 36). The pores of *H. abietinus* basidiocarps are also round to angular but aseriate and shallowly dentate with age (Figs. 38, 39). Dentations of the tube mouths and cracking of thin tube walls (30-40 μ) results in a labyrinthiform or sinuate configuration particularly in resupinate or effused-reflexed

basidiocarps. Thick-walled tubes (50-70 μ) produced behind the broad (0.6-1.2 mm), thick margin (greater than 1-2 mm) of *H. subchartaceus* basidiocarps are also round to angular (Figs. 40, 41) aseriately placed, shallowly dentate at their mouths but never tooth-like with age as in the basidiocarps of *H. pargamenus*. Irregular growth of tubes may be evident at the base of effused-reflexed basidiocarps, but the poroid configuration is observed in the younger, reflexed portions (Fig. 16).

Technical descriptions

Hirschioporus pargamenus (Fr.) Bond. & Sing.

Basidiocarp sessile, often narrowly attached (wedge-shaped) to dimidiate, effused-reflexed or resupinate, coriaceous-fibrous when fresh, rigid when dry but soon reviving; pileus 0-6 x 1-9 x 0.1-0.4 cm; surface white to tan or creamy, villose to velvety pubescent and concentrically sulcate, zonate; margin violaceous, narrowly sterile; context white, greater than 1 mm thick; pore surface white to violaceous, fading to brownish; tubes 1-3 mm long, seriate, mouths round to angular, 2-3 per mm, soon extensively tooth-like.

Hirschioporus abietinus (Dicks. *ex* Fr.) Donk

Basidiocarps sessile, solitary, imbricate or laterally confluent, attached at a point laterally or dorsally, dimidiate, effused-reflexed or resupinate, coriaceous when fresh, drying brittle, curving down when dry but soon reviving; pileus 0-6 x 1-3 x 0.1-0.2 cm; surface white to grey, strigose pubescent, more or less zonate, concentrically sulcate; margin violaceous, thin, narrowly sterile, even or undulating; context creamy to reddish brown when dry, very thin, less than 1 mm; pore surface white to violaceous, fading to dull brown colour; tubes 1-3 mm

long, aseriate, mouths round to angular, 2-3 per mm, thin-walled and shallowly dentate to sinuate.

Hirschioporus subchartaceus (Murr.) Bond. & Sing.

Basidiocarps sessile, dimidiate, solitary or laterally confluent, imbricate, effused-reflexed or resupinate, fibrous-coriaceous when fresh, drying rigid but quickly reviving; pileus 0-6 x 0.5-6 x 0.1-0.9 cm; surface white to gray with age, villose tomentose to hirsute at the base, inconspicuously zonate, sulcate; margin violaceous, thick and broadly sterile; context white to creamy, greater than 2 mm thick; pore surface white to violaceous, fading to brownish; tubes 1-3 mm long, aseriate, mouths round to angular, rather thick-walled, entire at first, shallowly dentate with age but never tooth-like, 2-3 per mm.

Discussion

The results of an examination of the macroscopic features of the basidiocarps of these species of *Hirschioporus* agree with the reports by Overholts (1953) and Bondartsev (1953). As pointed out by them, the basidiocarps of the three taxa are exceedingly variable with regard to macrostructure. This variability is responsible for most of the indecision associated with the identification of species limits based solely on gross morphology. Shape, size, colour and pubescence of basidiocarps have a range of expression that overlaps for each character in the three species, so that such features are not reliable in delimiting the taxa. Several basidiocarp shapes are characteristic of all three species and are produced as a result of position of the basidiocarp on the substratum. For example, the resupinate basidiocarps are found always on the underside of the log, whereas sessile basidiocarps are

collected on the sides of the log. Effused-reflexed basidiocarps are collected in an intermediate position. In response to the effects of gravity, the hymenial surface maintains a vertical orientation of tubes, providing optimal conditions for spore dispersal. This explanation agrees with the claim by Rhoads (1918) that position on the substratum is the primary factor dictating the shape of the sporophores of *Polyporus pargamenus* in nature. However, different positions on the log also entail differences in moisture (humidity) and light intensity which appear to be important factors affecting the size and colour of developing basidiocarps. Those of each species are small and deeply pigmented on the lower surface when produced in dry exposed habitats but larger and pale in moist, shaded sites. Brownish zonations, most conspicuous on the surface of *H. pargamenus* basidiocarps are produced as a result of alternating wet and dry growing conditions which affect the pigmentation of hyphae. The black, glabrous bands at the base of renewed basidiocarp growth can also be attributed to wounding and abrupt cessation in the growth of margins. This view is supported by the observations of "cremeous margins" reported for dried basidiocarps by Murrill (1907) and Rhoads (1918). Austwick (1968) and Taber (1966) in their general reviews of the factors which affect basidiocarp morphology have implicated light, temperature, and moisture. Kennedy and Larcade (1971) and States (1972) have pointed out the importance of these factors in the development of basidiocarps of *Polyporus adustus* and *Gloeophyllum saepiarium* in the field. Critical studies of the factors influencing the development of basidiocarps of *Hirschioporus* species in nature are yet to be done.

Degree of pubescence on the surface of basidiocarps is less variable for each species, although it is possible to find intergrading forms that

make this character less useful in the delimitation of species. At times, it is practically impossible to differentiate between the basidiocarps of *H. subchartaceus* and *H. abietinus* when the basidiocarps are young and small. Moreover, the description of this feature in a fashion that is meaningful to the inexperienced observer is very difficult. These studies indicate that pubescence is a quantitative character and is more explicitly expressed as thickness of surface trichoderm in relation to the thickness of the context. Therefore, the strigose pubescence of *H. abietinus* is much thicker than that of *H. pargamenus* (Figs. 30, 31), in vertical section, while the trichoderm of *H. subchartaceus* is intermediate in thickness (Fig. 32).

The age of basidiocarps is obviously a factor that must be considered in judging distinctions between species with respect to size, although the published descriptions do not clearly indicate such a consideration. Age also influences the expression of colour. The violaceous pore surface of all three taxa fades to a brownish colour (Fig. 33) with age, and the upper surface becomes greyish and zonate.

The difficulties in the distinction of these species with regard to the classical macromorphological characters of the basidiocarps is evidence for the close relationship that exists between them. Many of the characters that must be used for field identification are based largely upon tendencies that collectors have observed in "typical" specimens. For example, Overholts (1915) claimed that "with experience" he could distinguish the basidiocarps of *H. abietinus* and *H. pargamenus* on the basis of size and pubescence in general. However, I have found that the thin, brittle, reddish context of dried basidiocarps of *H. abietinus* distinguishes that species from *H. pargamenus* and *H.*

subchartaceus whose basidiocarps are thicker, white, and fibrous-coriaceous when dry. The distinction between the basidiocarps of *H. pargamenus* and *H. subchartaceus* is more of a problem. Both Overholts (1953) and Lowe and Gilbertson (1961) have indicated that the context of *H. subchartaceus* basidiocarps is thicker but I find this to be only generally true. However, as pointed out by these workers and Murrill (1907), the hymenial surface of the basidiocarps of *H. subchartaceus* remain poroid while that of *H. pargamenus* basidiocarps soon becomes conspicuously tooth-like. This distinction may not be obvious to the uninitiated collector because the pores of *H. subchartaceus* are shallowly dentate and become conspicuously tooth-like in thinner basidiocarps and at the base of effused-reflexed fruiting-bodies. Care must be taken to observe the young basidiocarps of the collection or the applanate specimens.

Variability and the similarity of intermediate forms of basidiocarps of these three species have caused mycologists to rely upon differences in substrate and geographic distribution. Apparent preferences exist in Western Canada but not in the whole of North America. Rhoads (1918) reported the occurrence of *H. pargamenus* and *H. abietinus* on the same *Tsuga* log in New York, while Macrae (1941) reported a collection of *H. abietinus* on *Betula* in Quebec whose monospore isolates were conspecific with other *H. abietinus* isolates. Although *H. pargamenus* and *H. subchartaceus* are confined to *Betula* and *Populus*, respectively in the West, they have been observed in the wood of the same species (*Prunus*, *Betula*) in Michigan. The three fungi have been collected in the same geographic locations in Western Canada and in close proximity. *H. pargamenus* is collected less frequently in the West probably because it demands the more humid environments characteristic of Eastern North America. Rhoads

indicated that *H. subchartaceus* was a western form of *H. pargamenus*. Overholts, and Lowe and Gilbertson also list it as part of the western polypores. However, it can be collected in Northern Ontario, in Michigan and in Iowa.

Overholts (1953) indicated a close relationship between these taxa and certain southern species with similar macroscopic and microscopic basidiocarp features. For example, *Polyporus versatilis* has a violaceous pore surface, thin context and conspicuously strigose pubescence. These characteristics suggest a close relationship to *H. abietinus*, but I have not examined basidiocarps of this species and thus cannot draw any conclusions on the basis of these features. *Polyporus sector* is described by Overholts as a "southern analogue of *P. pargamenus*" but again, first hand observations are required. From Overholts' account, this species lacks only the violaceous pore surface. These southern species possess microscopic hymenial features which are similar to those of the taxa found here. The significance of these features is discussed in Chapters III and VII. *H. pargamenus* is cosmopolitan, but *H. abietinus* is restricted to the Northern Hemisphere (Murrill, 1907). Cunningham (1965) does not include *H. abietinus* in the polypores of New Zealand. Also, *H. pargamenus* has not been reported in Sweden (Baxter, 1948) or the British Isles (Pegler, 1973; Cartwright and Findlay, 1958; Rea, 1922; Berkeley, 1860), although it is part of the mycoflora of other European countries. *H. subchartaceus* has only been reported from North America. A phytogeographic study of these species may be useful in elucidating evolutionary patterns on a world wide scale.

In conclusion it must be emphasized that macromorphological studies of the basidiocarps of *Hirschioporus* species need the support of other

information because of variability and the consequent intergradation of forms. Once supportive data are available, delineations can be made with greater confidence using characters such as context thickness and texture or pore configuration. The tendencies observed in other features can then be interpreted as differences in closely related taxa, and thus help to alleviate the practical problem of identifying these species.

TABLE 1. The number of collections* of *Hirschioporus* species observed on various substrates

Substrate	*H. pargamenus*	*H. abietinus*	*H. subchartaceus*
Betula	15	0	1
Populus	0	0	153
Prunus	1	0	1
Quercus	4	0	0
Acer	1	0	0
Fagus	1	0	0
Vitis	1	0	0
Picea	0	97	0
Pinus	0	21	0
Abies	0	2	0
Larix	0	1	0
Thuja	0	1	0

*Collections include both fresh specimens and herbarium material.

FIGURE 1. Distribution of *Hirschioporus pargamenus*, *H. abietinus* and *H. subchartaceus* in Western Canada.

○ *H. subchartaceus*

\+ *H. abietinus*

⊕ *H. subchartaceus* and *H. abietinus*

● *H. subchartaceus*, *H. pargamenus*, and *H. abietinus*

1

FIGURES 2-7. *H. pargamenus*.

FIGURE 2. Effused-reflexed basidiocarp. T592. X0.7

FIGURE 3. Applanate, imbricate basidiocarps. T360. X0.65

FIGURE 4. Imbricate, narrowly attached (wedge-shaped) basidiocarps. Note the concentrically sulcate and zonate surface. T592. X0.6

FIGURE 5. Resupinate (arrow) and centrally attached, circular basidiocarps. T592. X0.6

FIGURE 6. Sessile, narrowly attached basidiocarps. Lowe 3724. X0.5

FIGURE 7. Effused-reflexed, laterally confluent basidiocarps. T728. X0.8

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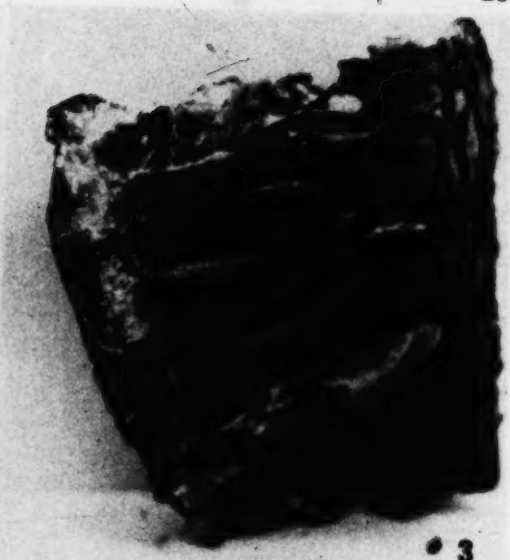

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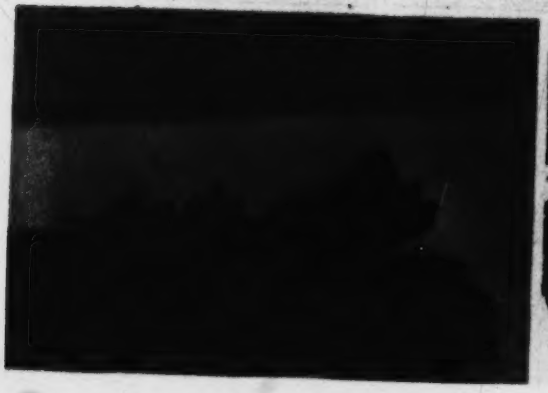

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FIGURES 8-13. *H. abietinus.*

FIGURE 8. Effused-reflexed basidiocarps. T54. X0.7

FIGURE 9. Pore surface showing thin-walled tubes with short projections and torn walls. DAOM 73846. X7.0

FIGURE 10. Effused-reflexed, imbricate basidiocarps which are laterally confluent. DAOM 73846. X1.0

FIGURE 11. Resupinate basidiocarps. T54. X1.0

FIGURE 12. Sessile, solitary basidiocarps. T269. X0.7

FIGURE 13. Pore surface of resupinate basidiocarp. Note sterile, growing margin. T54. X9.0

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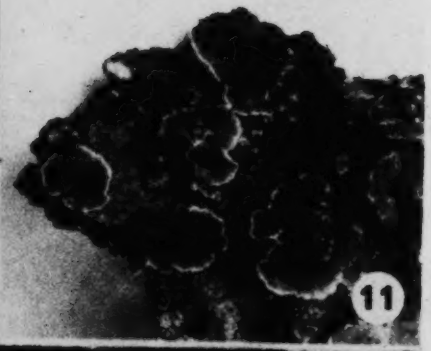
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FIGURES 14-20. *H. subchartaceus.*

FIGURE 14. Imbricate, broadly attached basidiocarps. T162. X0.6

FIGURE 15. Re-orientated basidiocarp showing renewed growth. T120. X0.8

FIGURE 16. Effused-reflexed basidiocarps. Note the tooth-like pore surface at the base. T368. X0.6

FIGURE 17. Broadly attached, basally effused basidiocarps. DAOM 5216. X0.5

FIGURE 18. Laterally confluent basidiocarps. T463. X0.7

FIGURE 19. Narrowly attached, sessile basidiocarps. DAOM 17876. X1.0

FIGURE 20. Resupinate basidiocarps. T22. X1.5

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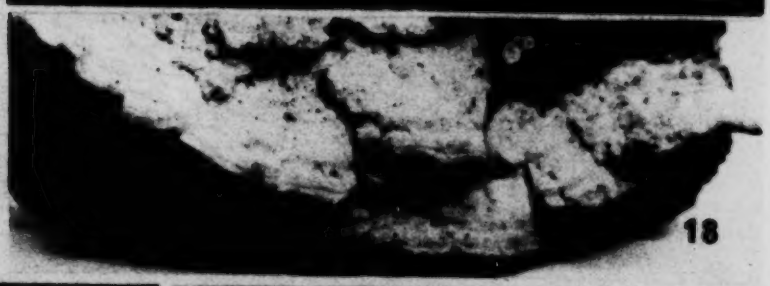
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FIGURES 21-29. Upper surface of basidiocarps.

FIGURE 21. Velvety-pubescent, inconspicuously zonate surface. *H. pargamenus*, T360. X2.3

FIGURE 22. Concentrically sulcate and zonate surface. *H. pargamenus*, DAOM 30472. X1.0

FIGURE 23. Blackish, glabrous zone. *H. pargamenus*, T360. X2.6

FIGURE 24. Strigose-pubescent, surface with concentric, blackish zones. *H. abietinus*, T412. X2.5

FIGURE 25. Sulcate and inconspicuously zonate surface. Note the curling down of dried basidiocarps. *H. abietinus*. DAOM 72305. X1.0

FIGURE 26. Strigose surface with black zones. *H. abietinus*, T244. X2.2

FIGURE 27. Villose-tomentose, concentrically sulcate surface. *H. subchartaceus*, T480. X1.9

FIGURE 28. Sulcate, inconspicuously zonate surface. *H. subchartaceus*, T480. X2.0

FIGURE 29. Black glabrous zones on the surface. *H. subchartaceus*, T480. X2.2

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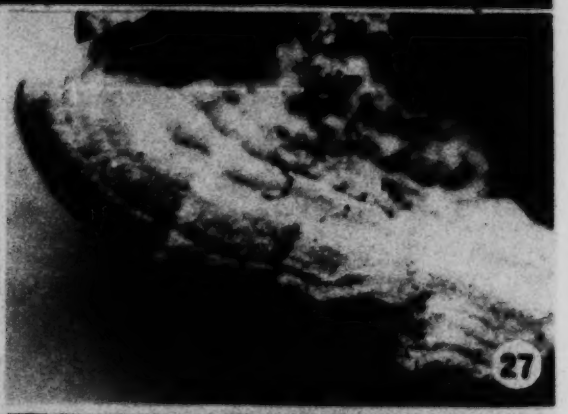
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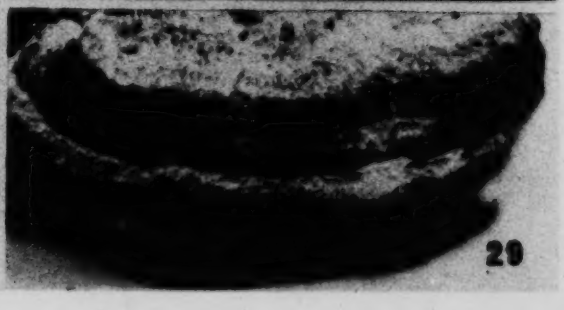
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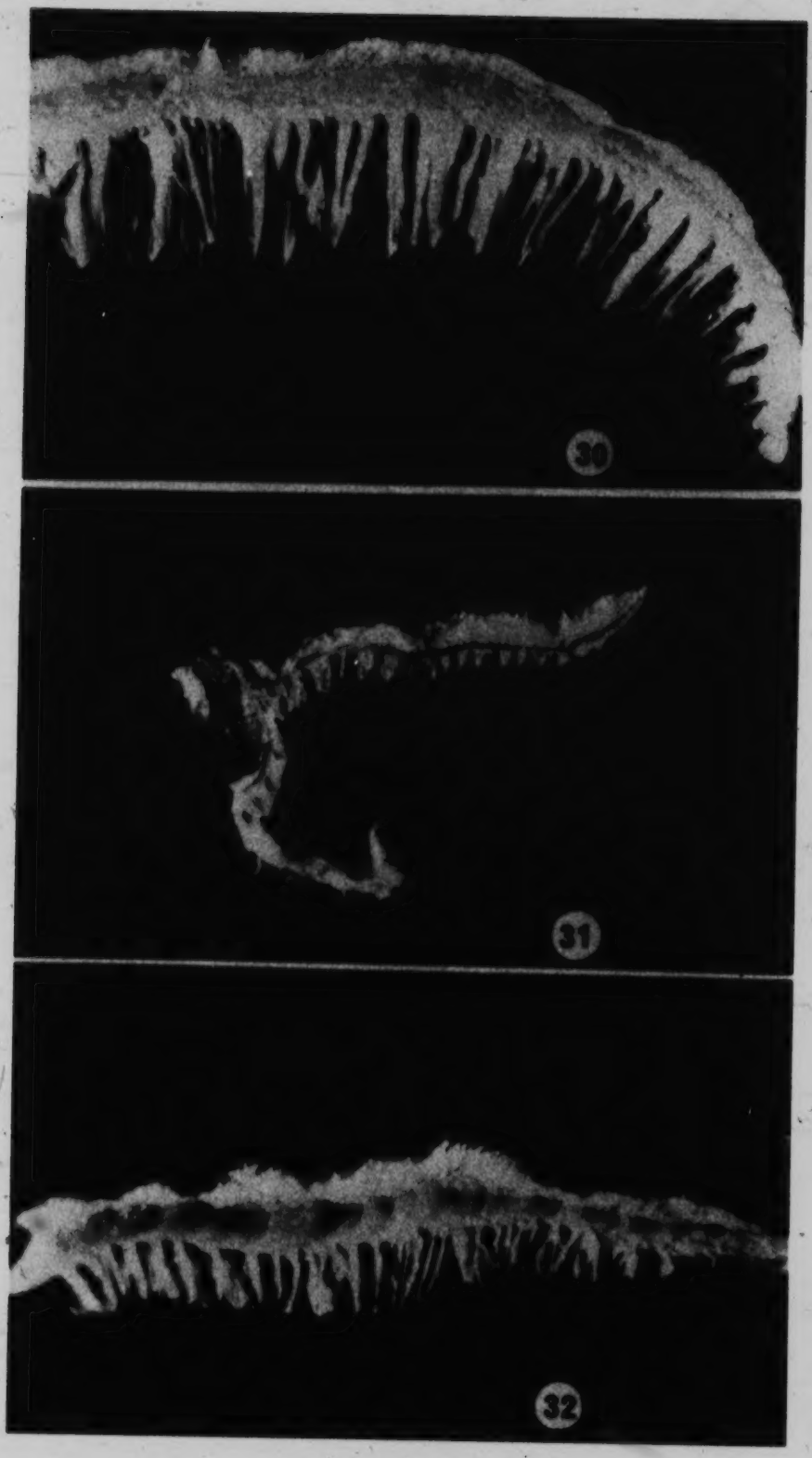
FIGURES 30-32. Vertical, median-longitudinal sections of basidiocarps.

FIGURE 30. Whitish context and narrow, pubescent layer on the surface. *H. pargamenus*, T592. X7.5

FIGURE 31. Thin-reddish, resinous context and thick, pubescent layer on the surface. *H. abietinus*, T130. X7.5

FIGURE 32. Thick-whitish context with reddish, concentric microzones and matted pubescent layer on the surface. *H. subchartaceus*, T338. X7.5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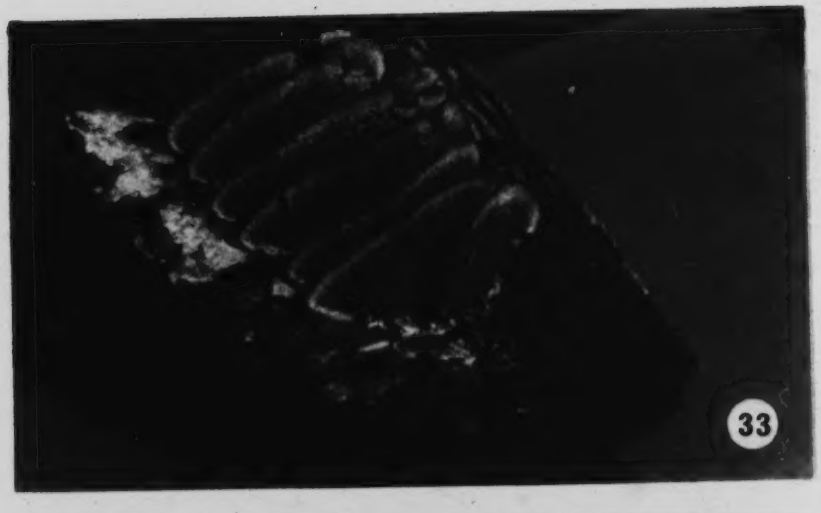
FIGURES 33-35.

FIGURE 33. Hymenial surface, violaceous at the margin but fading to brownish in older tubes. *H. pargamenus*, T360. X0.5

FIGURE 34. Violaceous to dark, brownish hymenial surface. *H. abietinus*, T93. X1.0

FIGURE 35. Violaceous hymenial surface. Note the intense pigmentation of sterile margins. *H. subchartaceus*. T24. X1.0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FIGURES 36-41. The hymenial surface of basidiocarps.

FIGURE 36. Tooth-like configuration with pores seriately arranged near the margin. *H. pargamenus*, Lowe, July 1931. X7.0

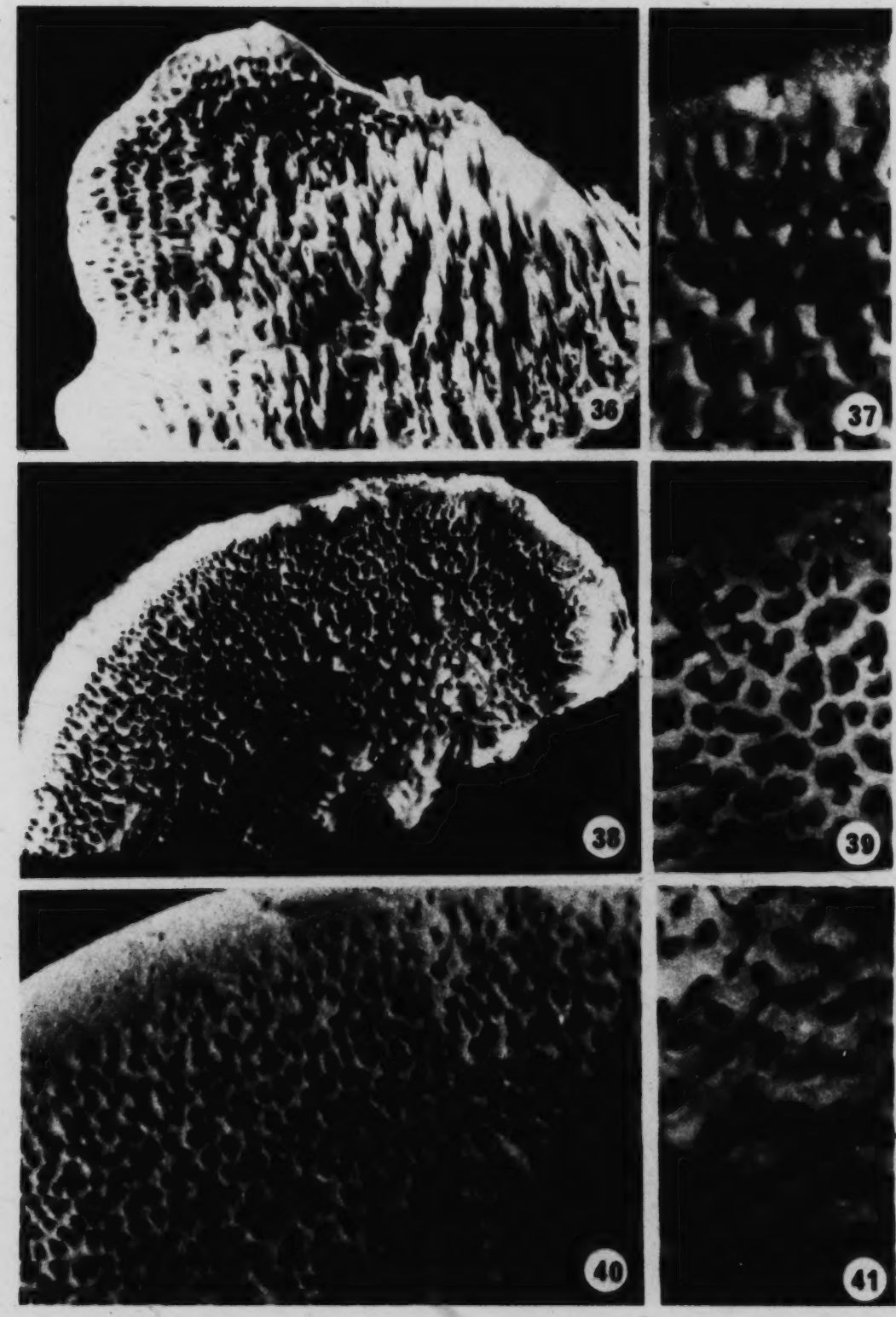
FIGURE 37. Mouths of young tubes. *H. pargamenus*, T592. X15.0

FIGURE 38. Poroid to shallowly dentate configuration. *H. abietinus*, T259. X7.0

FIGURE 39. Thin-walled tubes with short projections. *H. abietinus*, T48. X15.0

FIGURE 40. Poroid to shallowly dentate configuration with pores aseriately arranged. *H. subchartaceus*, RLG 6291. X7.0

FIGURE 41. Young, thick-walled tubes. *H. subchartaceus*, T595. X15.0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CHAPTER III

MICROSTRUCTURE AND DEVELOPMENT OF BASIDIOCARPS IN NATURE

Introduction

The use of microscopic characters which are usually less variable has been a welcomed approach to the taxonomy of polypores. These micro-morphological features are most often associated with the basidia, sterile hymenial elements (cystidia), and basidiospores, but may include hyphae found in the upper surface pubescence (trichoderm), context and tube walls (trama). Hyphal features include colour, shape, size, incrustation, wall thickness, branching, septation and presence or absence of clamp connections.

Overholts (1915a) reflected the opinion of many early workers when he stated that it was difficult to identify intermediate forms of *Polyporus pargamenus* and *P. abietinus* using external, macroscopic features of the basidiocarps. Yet, various mycologists who have described micro-morphology of hymenial and extrahymenial structures have met with the same degree of difficulty in differentiating the basidiocarps of these species (Rhoads, 1918; Bourdot and Galzin, 1928; Lowe, 1934, 1942; Overholts, 1953; Bondartsev, 1953). Their distinctions were based mostly on macroscopic features. For example, *P. subchartaceus* was distinguished from *P. pargamenus* on the basis of thickness of basidiocarp and configuration of the pore surface (Overholts, 1953; Lowe and Gilbertson, 1961). In the preceeding chapter, it was noted that such characters are often inconclusive and must be interpreted with caution.

In this case, the microscopic feature, spore size has proven to be a distinctive feature for the basidiocarps of *H. subchartaceus*, but as Overholts has pointed out, if basidiocarps are not in a sporulating condition then "the final evidence for distinguishing them is wanting".

Consistency and texture of the tissues of the basidiocarp have been characters of considerable importance to the taxonomy of polypores. With respect to the taxa considered in this thesis, it must be pointed out that texture of the context was used as a major macroscopic feature in the identification of the genus, *Hirschioporus*, proposed by Donk (1933). At that time, this feature was used by him to distinguish *Coriolus pargamenus* from *H. abietinus*. Consistency has seldom been related to microstructure. Ames (1913) is recognized as the first worker to systematically analyze basidiocarps of polypores and to describe the hyphal arrangement and orientation in the various tissues. With the aid of stained, thin sections she concluded that *Polystictus abietinus* did not fit Fries' concept of that genus. Her description of hyphal arrangement, like many others, is stated as "complex, interwoven hyphae". Corner was able to provide a much more detailed description of basidiocarp construction because he dissected out the components and studied its development (Corner, 1932a, 1932b).

The dissection method and the description of hyphal systems in terms of structure and function were major contributions to the studies of basidiocarp anatomy. Corner (1932a) described on the basis of structure and function three main types of hyphae in the basidiocarps and primordia of *Polystictus xanthopus*. Generative hyphae were described as "thin-walled, branched, septate, longitudinal or interwoven, 1.5-2.5 μ wide rarely 3 μ, with a clamp at each septum and abundant protoplasmic

contents". The skeletal hyphae were "thick-walled, unbranched, aseptate, straight or slightly flexuous, longitudinal, 2-5 μ wide, with the lumen more or less obliterated in mature parts, but the apices thin-walled with dense contents". The third type, binding hyphae, were described as "thick-walled, much branched, aseptate, interwoven, narrow, 1-2.5 μ wide, rarely 3 μ, with the lumen linear or obliterated in mature parts". Generative hyphae give rise to the skeletal hyphae which provide a framework for the basidiocarp, and to binding hyphae which tie it together. In his studies of the basidiocarps of *Fomes levigatus*, Corner (1932b) proposed three hyphal systems to describe basidiocarp construction: monomitic, with generative hyphae only, dimitic with generative and skeletal or generative and binding hyphae, trimitic, with all three types of hyphae. He described the tissues of *Fomes levigatus* basidiocarps as dimitic with skeletal hyphae. Although it was Corner who suggested that hyphal systems could be a key to the "natural" classification of polypores (1932a), it was Cunningham (1965) who applied this approach in his revised classification of the New Zealand Polyporaceae.

Microscopic studies of basidiocarp structure and development in the detail reported by Corner have not been reported for *H. abietinus* and *H. subchartaceus*. The results of Cunningham's studies of *H. (Trichaptum) pargamenus* using the concept of hyphal systems were briefly presented in a species description. A detailed hyphal analysis is required for a correct interpretation of basidiocarp structure and development according to Corner (1953) and Teixeira (1962). This is the approach used in the present studies where comparative, anatomical studies of the various stages of basidiocarp development are carried out for these three species. Objectives of such an investigation include an elucidation of additional

microstructural differences, a better interpretation of microstructural features previously reported, particularly hyphal structure, and characterization of the microstructural basis for macroscopic features.

Materials and Methods

Representative basidiocarps of each species collected at various localities were examined microscopically using the detailed approach outlined by Corner (1953) and Teixeira (1962). Fresh material or specimens that had been frozen were used. But, moistened herbarium material will yield similar results in terms of hyphal structure.

From longitudinal, freehand sections of primordia and young basidiocarps of a single collection, small squares (1 mm) of tissue were cut from the margin, upper surface, context and tubes. These squares of tissue placed on separate slides were flooded with a small drop of 5% KOH followed by a drop of 1% aqueous phloxine and then were teased apart with fine needles. Temporary mounts were obtained by gently flattening the preparation under a coverslip. As well, water mounts stained with phloxine, and lactophenol mounts stained with cotton-blue, were observed.

To determine hyphal arrangement, longitudinal and transverse sections (10-16 μ) of young basidiocarps were cut with a rotary microtome from material fixed in weak chrome-acetic acid and embedded in paraffin according to the procedures of Johansen (1940). The sections were stained overnight in an alcoholic solution of safranin and counterstained in fast-green (Jensen, 1962).

Freehand, longitudinal and transverse sections were examined directly

in KOH and phloxine for characterization of hymenial elements. As a rapid method of determining the types of hyphae small squares of tissue were macerated by gentle tapping of fresh mounts in KOH and phloxine. Line drawings were made with a camera lucida.

Observations

The basidiocarp development and resultant microstructure is very similar for each *Hirschioporus* species. Consequently, a description of the development from primordial stage to sporulating basidiocarp is provided for *H. pargamenus* as representative of this group. Differences from *H. pargamenus* as shown by the development and structure of *H. abietinus* and *H. subchartaceus* are noted with respect to margin, upper surface, context and tubes.

Development of *H. pargamenus* basidiocarps

(a) Primordia

Within the wood, the hyphae of *H. pargamenus* are narrow (1.5 μ), thin-walled, and branched with clamp connections at septae. Hyphae permeate the wood in all directions and emerge via cracks in the bark to produce small (1-2 mm), hemispherical tufts directly on the bark or on white, felt-like mats that fill bark fissures. The small, white tufts or primordia as they are termed here, are basidiocarp initials. On the sides of logs they continue to enlarge radially and horizontally to form sessile, shelf-like basidiocarps. At the 3-4 mm stage, a white, upper pubescent surface (called the trichoderm) and a violaceous, pore surface can be distinguished. Resupinate basidiocarps located on the underside of the substratum do not develop the trichoderm. They are narrowly

attached to the substrate at a point of hyphal emergence and increase in diameter by means of the radial and horizontal extension of hyphae in the growing margin.

The types of hyphae distinguished in the mats and primordia are identical to those observed in the sterile portions of developing basidiocarps. The margin of the tuft consists of a compact band (400 μ) of parallel, branching, thin-walled hyphae and unbranched, thick-walled cells. Within this band, thin-walled, generative hyphae (2.5-3.5 μ) with clamps (Fig. 43) give rise to longer, thick-walled, aseptate, unbranched and terminal, skeletal cells (Fig. 45). At a distance of 10-50 μ from the apex of the skeletal cell (90-1500 μ long), the walls begin to thicken gradually towards the base of the cell and this process continues until the lumen is nearly obliterated. The thick walls are non-staining and appear refractile. Behind the growing margin, a more interwoven construction is observed. Straight to slightly flexuous skeletal cells are entangled by branching, clamped, thick-walled, generative cells (20-200 μ long) with their lumina partly to almost completely occluded (Fig. 44). Thick-walled generative cells are distinguished from skeletal cells by their thickened clamps.

(b) Mature basidiocarps

Young basidiocarps of *H. pargamenus* continue to grow radially and horizontally by the extension of hyphal tips which contain yellowish, granular cytoplasm and occur in the peripheral, violaceous margin. This region is a thin (less than 1 mm), narrow, sterile band (0.2-0.6 mm) containing compact, thin-walled and thick-walled hyphae in a parallel arrangement (Fig. 42M). At a distance of 40-60 μ behind their growing apices, the walls of terminal cells are thickened gradually and terminal

skeletal cells are produced (Fig. 45). Skeletal cells (3.5-6.0 μ in diameter) are 200 to 1500 μ long. Although the apical portion of the cell is thin-walled, the older thick-walled portion is hyaline and refractile. A continuous front of growth is maintained by the forward extension of thin-walled branches of generative hyphae (Fig. 43) behind the skeletal cells.

The margin gradually merges into context (Fig. 42C). This context, approximately 600 μ behind the periphery of the basidiocarp, contains predominant, long, skeletal cells tightly interwoven by branching, thin- and thick-walled (Fig. 44) generative hyphae with clamps. Thickening of the multicellular, generative (non-growing) hyphae is frequently observed in this region of the basidiocarp. Lateral branches from the generative hyphae which have fallen behind the growing margin become thickened to their tips. Except for their narrow diameter and thickened clamps, they could be easily confused with skeletal cells which also become thickened to the tip in the context. The thin context (1.0-1.5 mm) of *H. pargamenus* remains fibrous and coriaceous. It is whitish in colour except for reddish-brown bands that are continuous with the coloured bands on the pileus surface. Hyphae in these areas, termed microzones by States (1972) in his studies of *Gloeophyllum saepiarium*, are incrusted by fine, yellowish granules.

The trichoderm (Lohwag, 1940), a pubescent layer on the upper surface of the basidiocarp is formed by the upward deflection (Fig. 42S) of thin-walled hyphae and skeletal cells in the margin and young context. Concentric sulcations are ridges of loosely entangled hyphae alternating with bands of shorter matted hyphae. The velvety pubescence of *H. pargamenus* is composed of short (100 μ), erect skeletal cells and

generally collapsed generative hyphae. Longer, appressed hyphae in aggregates give some basidiocarps a silky to glabrous appearance. The creamy colouration with brownish, concentric bands is associated with hyphae containing pigmented contents, while the blackish, glabrous zones contain incrusted hyphae in the upper context and surface.

The violaceous, poroid, hymenial surface is formed by the downward deflection (Fig. 42) of hyphae 0.2-0.6 mm behind the margin. This area is called the pore-field (Corner, 1932b) and contains ridges of interwoven, branched, generative hyphae and skeletal cells which make up the tube walls (Fig. 42T). The much interwoven arrangement of branched, thick- and thin-walled hyphae in the lower context where hyphae have changed their direction of growth, gives way to a pattern of parallel, thick-walled hyphae in the tube walls. As in the margin of the basidiocarp proper, the edge of the tube is the site of growth of generative hyphae and the thin-walled tips of skeletal cells. Tubes of *H. pargamenus* soon become conspicously tooth-like as a result of the irregular extension and tearing of thin (30-50 μ) pore walls. Thickened, narrow (2.8-3.0 μ), short (up to 180 μ), sparsely branched, terminal and intercalary cells of the generative hyphae are frequently observed in the trama (Fig. 44). Occasionally, skeletal cells and thickened laterals of generative hyphae have swollen, thick-walled tips (Fig. 46) and slight indentations indicative of limited renewed growth (Fig. 47). Thin-walled hyphae in the trama grow outward and branch repeatedly to form a compact palisade of end cells lining the tube walls. Below the hymenial layer the generative hyphae display crystalline incrustations on their surfaces. The hymenium contains thin-walled, clavate (Figs. 48, 49) basidia (11-16 x 4-6 μ) and clavate to bluntly pointed cystidia

(10-15 x 4-6 μ) which are incrusted at the tip by fine, hyaline crystals (Fig. 52B). Cystidia project 7-10 μ above the hymenium and are more rarely thick-walled (Fig. 52A). Sterile, terminal clavate cells are sometimes observed to possess hyphoid projections (Fig. 51) but lack incrustation. Basidiospores are hyaline, thin-walled cylindrical to slightly curved and 5-6.5 (-7.4) μ long, 1.8-2.2 μ wide (Fig. 50). Broad, obtuse lateral projections into the lumen of pores in cross sections of the hymenial surface were observed. These peg-like structures consist of compact, parallel hyphae and have hymenial layer at their base in serial section.

Distinctions shown by basidiocarps of *H. abietinus* and *H. subchartaceus*

(a) Margin

Although the margin of *H. abietinus* basidiocarps is similar to that of *H. pargamenus*, the basidiocarp margin of *H. subchartaceus* is usually thicker (greater than 1 mm) and broadly sterile (0.6-1.2 mm). This thick margin contains a greater proportion of thin-walled generative hyphae than are observed in the margin of the other species.

(b) Context

When dried, the thin context (less than 1 mm) of *H. abietinus* basidiocarps becomes brittle and reddish in colour. In KOH-phloxine mounts, yellowish granular incrustations cement together the compacted, parallel hyphae and account for the resinous texture. The thicker (greater than 2 mm) context of *H. subchartaceus* remaining fibrous-coriaceous and whitish in colour, contains loose strands of thick- and thin-walled hyphae interwoven by thin-walled, clamped hyphae. Reddish-brown microzones containing incrusted hyphae are observed in mature

basidiocarp context (Fig. 32, Chapter II).

(c) Trichoderm

Compared to the surface of *H. pargamenus* basidiocarps, the white, strigose pubescence of *H. abietinus* basidiocarps consists of longer fascicles of tangled skeletal cells (800-1200 μ) loosely entwined by branched, thick- and thin-walled, collapsed generative hyphae. Like the pubescence of *H. abietinus*, the trichoderm of *H. subchartaceus* basidiocarps consists of fascicles of long, skeletal cells and generative hyphae but the aggregates of hyphae are more matted and entangled.

(d) Tubes

The pore surfaces of *H. abietinus* and *H. subchartaceus* are not characterized by tooth-like tubes as in *H. pargamenus*, but they do have shallow dentations. Microstructurally, the trama of the hymenial surface of the three taxa is similar. But, thick-walled cystidia are very frequent in the hymenium of *H. abietinus*. Basidiospores of *H. abietinus* are not distinct from those of *H. pargamenus* in size (Table 2), while those of *H. subchartaceus* are larger, being 7.4-9 (-11) x 2.0-2.5 μ.

Discussion

As pointed out in the reviews of Lentz (1954, 1971) and Smith (1966), extensive modifications are characteristic of the hyphae in basidiocarps of polypores. The taxonomic significance of these hyphal modifications, found in all parts of the basidiocarp, has been discussed by Teixeira (1962) and Donk (1964). Hymenial elements received most of the attention of early taxonomists and anatomists who considered the micro-morphology of polypore basidiocarps. Descriptions of hyphal anatomy included wall

thickness, branching, presence or absence of clamps and the relative arrangement of hyphae in the basidiocarp. Generally, these observations were made of fragments of hyphae in thin-sections without consideration of their development. Detailed studies of basidiocarp structure, development and hyphal anatomy were initiated by the careful investigations of Corner who was able to contribute a better interpretation of the form and function of various hyphal modifications.

An awareness of the hyphal diversity in the basidiocarps, particularly in the hymenium, of these species of *Hirschioporus* is evident in the taxonomic studies of Overholts (1953) and Bondartsev (1953). Of the many workers who have described microscopic features in the basidiocarps, Bondartsev provides the most detailed resumé for *H. abietinus* and *H. pargamenus* in Europe. The work of Overholts on North American taxa has been selected because it includes the three species in a detailed comparison of microscopic as well as macroscopic features of the basidiocarps. As previously stated, microscopic examination by these authors is largely focused on the hymenial elements, but Bondartsev presents a detailed observation of surface and context hyphae and their arrangement. The description of *Trichaptum pergamenum* by Cunningham (1965) provides an interesting basis for comparison because it reflects the "hyphal systems" approach used by Corner to analyze basidiocarps.

The results of developmental and morphological studies of microscopic structures in the basidiocarps of *Hirschioporus* species are discussed with respect to the published observations by Overholts (1953), Bondartsev (1953) and Cunningham (1965). Hymenial features are dealt with first, and are followed by consideration of structures other than elements of the hymenium.

The hymenium of these three taxa is a compact layer of modified terminal cells of branching thin-walled hyphae. In general, the basidia, cystidia and their immature stages do not provide features by which these fungi can be distinguished. Cunningham (1965) has described the hymenium of *T. pergamenum* as "30 μ deep, a dense palisade of basidia, paraphyses and metuloids". The term, "metuloid" is used by him to describe cystidia, while "paraphyses" refers to immature basidia and cystidia. However, I raise objection to the use of these two terms in description of the structures observed in the hymenium of *Hirschioporus* species. Immature basidia and cystidia are unlike the sterile, hyphoid structures commonly termed paraphyses in the hymenium of ascomycetes. If they must be named, the terms "basidioles" and "cystidioles" (Lentz, 1954) are more applicable. However, immature basidia lacking sterigmata and immature cystidia lacking crystals are practically indistinguishable except for the blunted points on the latter elements.

The occasional observation of terminal cells with elongate, hyphoid tips and projections suggests that these immature elements may be capable of growth under moist, storage conditions. Cunningham's definition of metuloids as "ancillary organs formed from the terminal ends of hyphae" does not suit the capitate-incrusted cystidia which are distinct cells attached to generative, thin-walled hyphae in the undifferentiated subhymenium. Therefore, the use of the more general term, cystidium to describe sterile crystal-incrusted elements in the hymenium of *Hirschioporus* basidiocarps is recommended.

Contrary to the reports of Cunningham and Overholts, thick-walled cystidia were observed for these species. In agreement with the observations of Bondartsev (1953), they are encountered more frequently

in the basidiocarps of *H. abietinus*. Lentz (1971), in his review of the reports of thick-walled cystidia in *Lopharia crassa* (Hydnaceae), has emphasized that these cystidia must not be confused with thick-walled tips of hyphae protruding into the hymenium. I have observed that thick-walled cystidia can be distinguished from such hyphae observed in the hymenium of *Hirschioporus* species by tracing them to their attachment to the generative hyphae and by noting that thick-walled hyphal tips lack crystalline incrustations.

Crystals on cystidia have been identified as calcium oxalate by both Overholts and Bondartsev. Lentz (1954) reports that Patouillard had observed calcium oxalate crystals as early as 1882 on the walls of cystidia in *H.* (*Polyporus*) *abietinus* and had proposed the theory, held by many modern mycologists (Smith, 1966), that cystidia are excretory elements. He further cites authors who have observed cystidia with thin-walled tips, apical pores and mucilaginous caps in other species. These observations are considered as support for the theory that cystidia have an excretory function. Calcium oxalate is commonly reported as a bi-product of fungal metabolism deposited as crystals on the surface of cystidia in a variety of species (Lentz, 1954). Excretion of this toxic substance requires chemical confirmation. Refractile caps and papillae were personally observed beneath the crystals on the cystidia of *Hirschioporus* species. Capitate-incrusted, cystidia-like branches were observed on the aerial hyphae of the vegetative mycelium in culture (Chapter IV). Nutritional studies and examination of cystidia with the electron microscope are suggested as a source of information that might support the excretory theory and provide fundamental characteristics which distinguish cystidia from

basidia.

The larger basidiospores of *H. subchartaceus* were a good microscopic distinction between that species and the other taxa whose spores are indistinguishable. These spore measurements agree with published reports by Overholts (1953) and Lowe and Gilbertson (1961) who used this key feature.

The search for morphological features of the hyphae in the extrahymenial tissues of the basidiocarps which could serve as characteristics by which the three *Hirschioporus* species might be distinguished has been fruitless. The basidiocarps and the primordia of the three fungi are constructed in the same manner of three types of hyphae. Most obvious are the long, unbranched, terminal, thick-walled, skeletal cells. These cells which are arranged in a more or less parallel manner, are interwoven by thin-walled, branched generative hyphae consisting of many, shorter cells delimited by septae and clamp connections. These hyphae give rise to the skeletal cells in the growing margin, but in the older parts of the basidiocarp the generative hyphae develop uniform thickening of the walls of intercalary cells and lateral branches. These hyphae are distinguished by their shorter cells and thick-walled clamps.

There are no published descriptions of the hyphal anatomy of *H. subchartaceus* basidiocarps for comparison with the findings in this thesis. However, Overholts (1953) and Bondartsev (1953) have given brief descriptions of the structure of hyphae in the basidiocarps of *H. pargamenus* and *H. abietinus*. They recognized parallel, "thick-walled, simple" (Overholts, 1953) and "solid hyphae" (Bondartsev, 1953) in the basidiocarps. Although Overholts did not mention thin-walled hyphae for

these species, Bondartsev reported the occurrence of thin-walled and branching hyphae. Clamp connections which are the identifying feature of generative hyphae have been reported as "inconspicuous" in *H.* (*Polyporus*) *pargamenus* basidiocarps (Overholts, 1953) and "s[illegible]e" by Bondartsev (1953) for the same species. Thickening of generative hyphae was not reported by either worker. The failure of these workers to see clamp connections may have been due to their methods of observing the basidiocarps and the material that they used. For example, thin-walled clamped hyphae are not obvious in serial sections of basidiocarps, and often the generative hyphae are collapsed and disintegrated in old herbarium specimens.

Moreover, Overholts and Bondartsev did not identify types of hyphae detected by Corner (1932a,b) in his detailed dissection of the basidiocarps of *Fomes levigatus* and *Polystictus xanthopus* because they examined only hyphal fragments. Cunningham (1965), on the other hand, used the hyphal systems concept in his description of the basidiocarps of *T.* (*Hirschioporus*) *pergamenum*. For this taxon, he has recognized two types of hyphae in the basidiocarp: "skeletal hyphae 3-4.5 μ diameter, walls to 1 μ thick, sparsely branched, aseptate, tending to collapse; generative hyphae 2.5-3 μ diameter, walls 0.5 μ thick to 1 μ in context hyphae, branched, septate, with clamp connections". This description includes the three types of hyphae that I have detected in the basidiocarps of the three fungi. The generative hyphae with walls 1 μ in thickness correspond to the thickened generative hyphae that I have observed. However, I cannot substantiate Cunningham's observation of "collapsed" skeletal hyphae, and find the possibility of doing so unlikely if the definition that they have thickened walls is adhered to. Macrae and

Aoshima (1966) in their studies of the basidiocarps of *H. laricinus*, a close relative of *H. abietinus* (Macrae, 1967) have recognized a dimitic construction consisting of skeletal and generative hyphae.

Corner's terminology for the different types of hyphae that he observed in a limited number of polypore basidiocarps is simple and has appealed to many mycologists studying basidiocarp anatomy. However, these terms have been almost dogmatically accepted and too many researchers have attempted to mold their description of deviant hyphal structures into his restrictive concept of hyphal systems.

Skeletal hyphae are assigned a structural role in providing the framework for the basidiocarp, while generative hyphae give rise to the modified hyphae and differentiated terminal cells. In *Hirschioporus* species there are thick-walled generative hyphae which have both a generative and structural role in basidiocarp development. Moreover, when intercalary cells subtending skeletal cells become thick-walled in the older context, long, thick-walled hyphae with thickened clamps are produced and thus confuse the identification of the unicellular, aseptate skeletal elements. The thickening of the walls of hyphae with clamps does not indicate that they have lost their generative function. I have found that in the region above the tubes, these hyphae in the context of *Hirschioporus* species are capable of renewed growth. Corner, himself, has made similar observations for thick-walled hyphae of *Fomes levigatus* (1932b) and makes the following statement: "Moreover, there are irregularities in the development of generative hyphae, for some of them stop growing and their walls thicken up to the apex, and then delicate colourless processes are frequently extruded which grow on as ordinary generative hyphae and apparently contribute to the hymenium". Furthermore,

Corner (1953) indicated that the form of the differentiated elements is not entirely stable in his statement that in the basidiocarps of *Pterula* (Clavariaceae), the "skeletals return after a long period of skeletal growth to the state of generative hyphae". These examples of "irregularities in deportment" support my view that it is more important to precisely describe what is actually observed rather than attempting to fit the hyphae into Corner's structural and functional terminology which may not be applicable.

A more important aspect in the identification of hyphal types is their origin and development in the basidiocarp. Briefly, the growing regions of the basidiocarps of *Hirschioporus* species are the margin proper and the sterile edge of tubes. Thin-walled, generative hyphae in the growing regions give rise to terminal cells and lateral branches which grow forward and contribute to the thin-walled hyphae in the margin or become thick-walled skeletal cells. Behind the margin the unbranched, aseptate, skeletal cells are bound by thick- and thin-walled, branched, generative hyphae consisting of many cells. Hyphae grow upward at the margin to form a pubescent surface containing skeletal cells and generative hyphae, and downwards to form the ridges of the pore-field. In these ridges growth in a downward direction is continued while generative hyphae grow outward in the tube walls and branch repeatedly to form the hymenial layer. Therefore, in the growing regions of the basidiocarps, the generative hyphae are recognized by their thin walls, branching, and most important, their clamp connections. The formation of skeletal cells from these fundamental hyphae is the result of differentiation of terminal cells in the margin. Two processes are going on simultaneously: the cell is extending by the growth of a thin-walled

apex containing dense cytoplasm, while behind the apex the walls are thickening towards the base of the cells. Because I have observed that thin-walled, terminal cells must reach a certain length (approximately 200 μ) before thickening begins, I have postulated that thickening and apical growth proceed until a maximum amount of wall thickening slows down the rate of forward extension of the terminal cell. This thickening may trigger the initiation of lateral branches and their increased rate of growth into the marginal front where the same thickening and growing processes are repeated. The skeletal cells left behind the growing margin become thickened to their tips and provide the framework for the basidiocarps. Behind the margin, long, unbranched skeletal cells thickened to the tip are frequently observed in the basidiocarps of *Hirschioporus* species. In his discussion of the formation of skeletal hyphae in *Gloeophyllum saepiarium* basidiocarps, States (1972) has suggested that wall thickening may limit the translocation of nutrients to the growing apex thereby interrupting an apical dominance situation.

The thick-walled, generative hyphae in the older tissues of the basidiocarps of *Hirschioporus* species are formed in a different manner from the thick-walled, skeletal cells. The walls of non-growing intercalary cells and lateral branches become uniformly thickened. Various stages of thickening are seen from partial occlusion of the cell lumen to almost complete obliteration. Thickening of generative elements in older tissues only exemplifies the importance of location in the development of morphology. In this regard, Edwards (1972) cites the binding elements of *Coriolus hirsutus* and *C. pubescens* as examples of microstructures which have attained their much-branched form through their "intrusive growth" in the context of the basidiocarp. Moreover, the

factors which govern the morphogenesis of terminal cells in the margin and in the hymenium must certainly be complex. The generative hyphae are the source of both types of cells, but Donk (1971) states that skeletal cells in the margin are under the influence of "epigenic factors" different from those affecting the development of cystidia and basidia in the hymenium. These factors, essentially micro-environmental in his conception of the situation, must be the same for basidia and cystidia which are produced on the same hyphal segment in the basidiocarps in *Hirschioporus*. I have observed that a cystidium occupies a terminal position (Fig. 52C) which may place it under the influence of different internal factors such as nutrition. Further studies of these modified cells in terms of development and its controls are much needed for a correct interpretation of the features which have taxonomic significance. In other words, it is necessary to know whether a cystidium (thin-walled) is a discrete element or a modified basidium, and whether a thick-walled cystidium is a modified skeletal cell.

Corner's use of the term, "skeletal hypha" reflects a different concept of the development of these structures than what is described here. He believed that thin-walled generative hyphae were transformed into thick-walled skeletal hyphae by the loss of ability to branch and produce septae. He named an intermediate stage, "mediate hyphae" whose walls were not as thick as the mature skeletal hyphae. These elements were aseptate and terminal in his definition. Because Corner has failed to recognize the development of these structures as growing terminal cells, I have chosen to abandon the term "hypha", and have called them skeletal cells in order to emphasize their development and morphology. The term hyphae has been reserved for those structures which are

multicellular. Smith (1967) and Edwards (1972) have adopted a similar stand, although they employed the term "skeletal element". I do not accept the use of the term "mediate hyphae" to describe the thin-walled, terminal cells in the margin of *Hirschioporus* basidiocarps. These cells must be designated as generative cells which may become skeletal cells. This view is slightly different from the one held by Edwards (1972) who depicted terminal, thin-walled cells in the margin of *Coriolus hirsutus* basidiocarps, but claimed that there were no generative hyphae in the margin and that all terminal cells become skeletals in this region of the basidiocarp. In the basidiocarp margin of *Hirschioporus* species, generative hyphae are very common and probably contribute to the ease with which it is wounded upon sudden dessication. The exact limits of the margin are hard to define, but in any case, generative hyphae by my definition have been observed in a terminal position.

In view of the complexities of hyphal and basidiocarp development the mere categorization of basidiocarp construction as monomitic, dimitic or trimitic is of little taxonomic significance. For separating these three taxa it is of no use. However, the similarities in the development of the various microstructures and the basidiocarp as a whole for the three *Hirschioporus* taxa is good evidence for their close relationship. A better understanding of the details of basidiocarp structure and the development of it is essential to the delimitation of taxa and recognition of generic relationships. The restrictions of the "mitic" idea in describing the varied constructions of polypore basidiocarps are illustrated by the findings of Corner, himself. For example, he recognized two dimitic systems: one with generative hyphae and skeletal cells, and another with generative hyphae and binding cells.

Moreover, in the basidiocarps of *Polyporus betulinus*, Corner (1953) has reported that the young flesh is monomitic for some time, and then, the context becomes dimitic with skeletal hyphae and the tubes dimitic with binding hyphae. Consequently, taxonomic decisions based on the mitic system alone are extremely risky. The dimitic system of basidiocarp construction that I have reported for *Hirschioporus* species is very similar to the construction of the basidiocarps of *Cerrena unicolor* which has been reported as monomitic with hyphae becoming thick-walled (van der Westhuizen, 1963). Yet, significant differences are seen in the development of basidiocarps and component hyphae. He reported the occurrence of terminal, thick-walled, sparsely branched, septate hyphae with thickened clamps and considered them analogous to Corner's skeletal hyphae. They are unlike the longer, terminal, unicellular thick-walled structures that I have termed skeletal cells in the basidiocarps of *Hirschioporus* species.

Although the terms that Corner has proposed to describe hyphae in basidiocarps are somewhat limiting in their application to every polypore, they are well established in studies of basidiocarp anatomy. Rather than erect a different terminology, these terms, which have a certain degree of historical priority have been utilized in this thesis with slight modification. Greater emphasis must be placed on the need for more careful examination than on the need for new terms.

Although there are no species distinctions between the various hyphal components of the basidiocarps, their arrangement and relative abundance are helpful in describing macrostructural features which have been used with some caution in the distinction of *Hirschioporus* species. For example, the strigose-pubescent, upper surface of the basidiocarps of

H. abietinus consists of loosely interwoven, thick- and thin-walled hyphae or coarse hyphal fascicles, while the velvety pubescence of *H. pargamenus* basidiocarps consists of shorter, more tightly entangled or erect skeletal and generative hyphae. The resinous texture of the context of *H. abietinus* basidiocarps as distinguished from the fibrous-coriaceous context of *H. pargamenus* and *H. subchartaceus* expresses the difference in arrangement of hyphae and the relative abundance of the types. In the *H. abietinus* context, the hyphae (mostly thick-walled, skeletal cells and thickened generative hyphae) are compact and more or less parallel, while the context of the other species contains strands of skeletal cells and thick- and thin-walled generative hyphae loosely interwoven by abundant, thin-walled generative hyphae.

I have found that the reddish-brown resinous context of *H. abietinus* contains hyphae incrusted by a yellowish granular substance. This granular material is found also in the microzones of the context, in the surface zonations and in the wounded margins of *H. pargamenus* and *H. subchartaceus* basidiocarps and its presence indicates a close relationship between these two fungi and *H. abietinus*. Austwick (1968) claims that coloured bands in polypore basidiocarps are associated with "accumulated protoplasm in the hyphal tips, where pigment (especially that showing on hyphal death) is likewise concentrated". In *Hirschioporus* basidiocarps black glabrous zones contain hyphae with granular incrustations.

The violaceous pigmentation seen in the growing regions of the basidiocarps by reflected light, is localized in the thin-walled compact hyphae which show a greater concentration of protoplasm. Yellowish granules which are observed within the cells by transmitted light may be responsible for the pigmentation. Incrustations of hyphae (usually empty

and thin-walled) in the microzones, on the surface and beneath the hymenium, may be this same material. Preliminary attempts to characterize the pigment involved the gentle refluxing of ground, dried, marginal and tube tissues of *H. subchartaceus*, where violaceous pigmentation is strongly developed, in 2% HCl in iso-butanol. A yellowish-brown extract produced a single, yellow spot when separated by paper chromatography in two dimensions using in the first, a mixture of tertiary butyl alcohol, acetic acid, and water (3:1:1) and then, 15% acetic acid in water. It must be emphasized that these experiments are insufficient evidence from which to draw conclusions concerning the nature of the pigment. Since the substance may have been altered during the isolation, different solvent systems need to be tested and compared for all three species. Chemotaxonomic studies are complicated by variability within populations and strain differences in culture (Tyler, 1971). Consequently, a proper analysis of pigments in these species would be interesting but is a study in itself. The chemistry of polypore pigments is not well known. Birkinshaw (1965), Arpin and Fiasson (1971) indicate that many colourants are derivatives of para-diphenyl benzoquinones such as polyporic acid and thelephoric acid responsible for the brownish, blackish or violaceous colours in the basidiocarps of *Phaeolus rutilans* and *Thelephora* species. The macroscopic similarities and dissimilarities in the pigmentation of *Hirschioporus* species vary with age and environment. The specific colour may not be a qualitative character but a quantitative difference in the relative proportions of several substances responsible for pigmentation.

In summary, microstructural analysis of basidiocarps confirms the decisions arrived at by the traditional, macroscopic approach, that these

are closely related species. The morphology of hyphae and cells does not provide many characteristics by which the species can be distinguished. Spore size is a distinguishing feature but it applies only to *H. subchartaceus* and *H. pargamenus*. The greatest value of microstructural analysis lies in its more precise description of macrostructural features in terms of relative abundance and organization of hyphal types. Detailed developmental studies are of primary importance in the identification of the various hyphal components and in the description of the construction of the basidiocarp. For these species, the pattern of basidiocarp development has taxonomic value in demonstrating relationship at the generic level. I believe that a developmental approach to the study of polypores is important for the detection of relationships between species in other genera and for the classification of these genera into families. Such an approach is likely to prove more useful than a description of the basidiocarp in terms of the "mitic system". Analysis of fruiting-bodies produced in the field provides one kind of information concerning the developmental morphology of these fungi. This information must be supported by studies of basidiocarps and vegetative mycelium in culture where the fungi are grown under standard conditions.

TABLE 2. Size of microstructural elements in basidiocarps produced in the field

	H. pargamenus	*H. abietinus*	*H. subchartaceus*
Generative hyphae	2.5-3.5 µ	2.4-3.5 µ	2.5-3.5 µ
Skeletal cells	3.5-6.0 µ	3.0-6.0 µ	3.5-6.0 µ
Cystidia	10-15 x 4-6 µ	12-18 x 4-6 µ	12-18 x 5-7 µ
Basidia	11-16 x 4-6 µ	12-16 x 4-6 µ	12-16 x 5-6 µ
Basidiospores	5.0-6.5 (-7.4) x 1.8-2.2 µ	5.6-6.3 (-7.4) x 2.1-2.7 µ	7.4-9.0 (-11) x 2.0-2.5 µ

FIGURE 42. A diagramatic representation of the arrangement of hyphae in a developing sessile basidiocarp of *H. pargamenus*.

M - margin

T - tubes

S - surface

C - context

Wall thickening is designated by solid, black lines.

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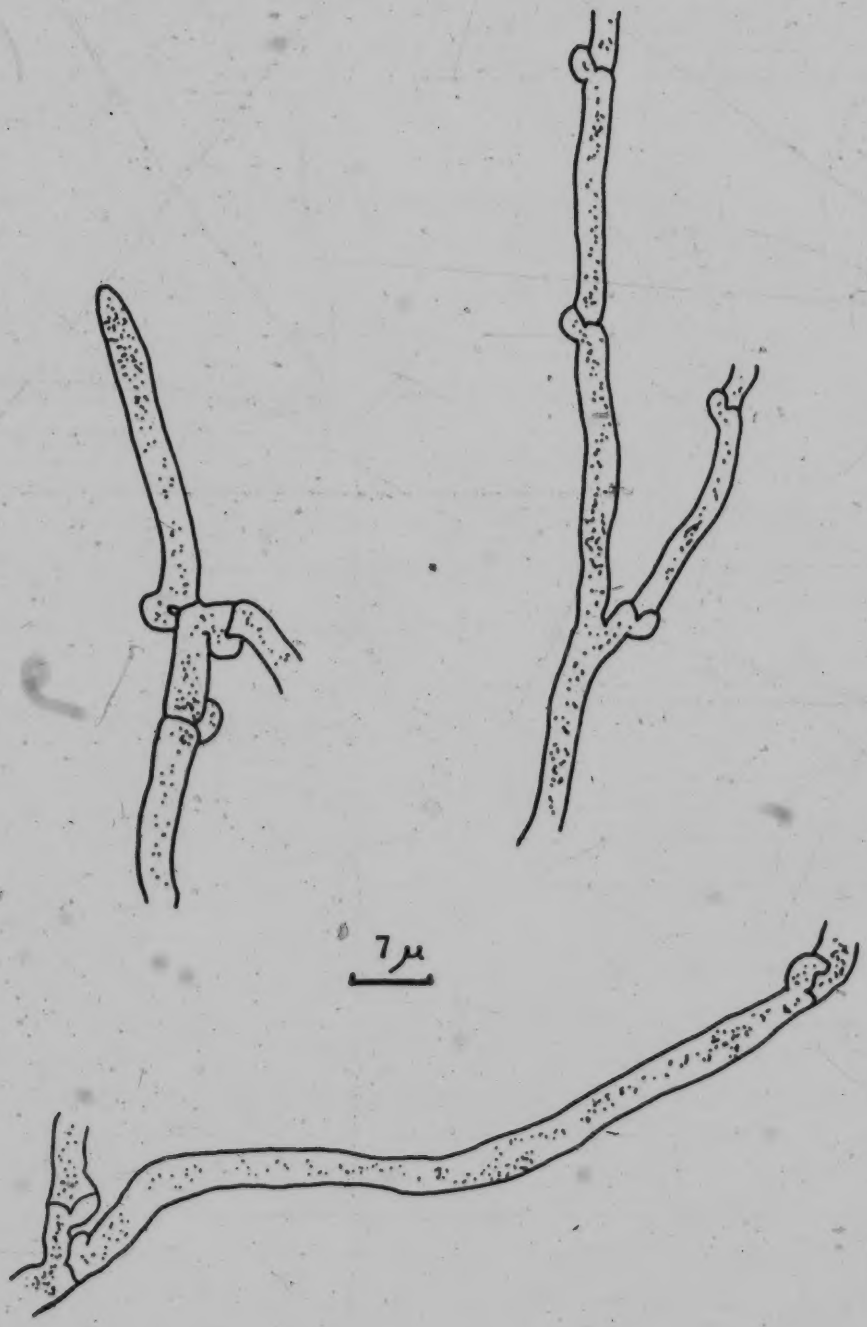
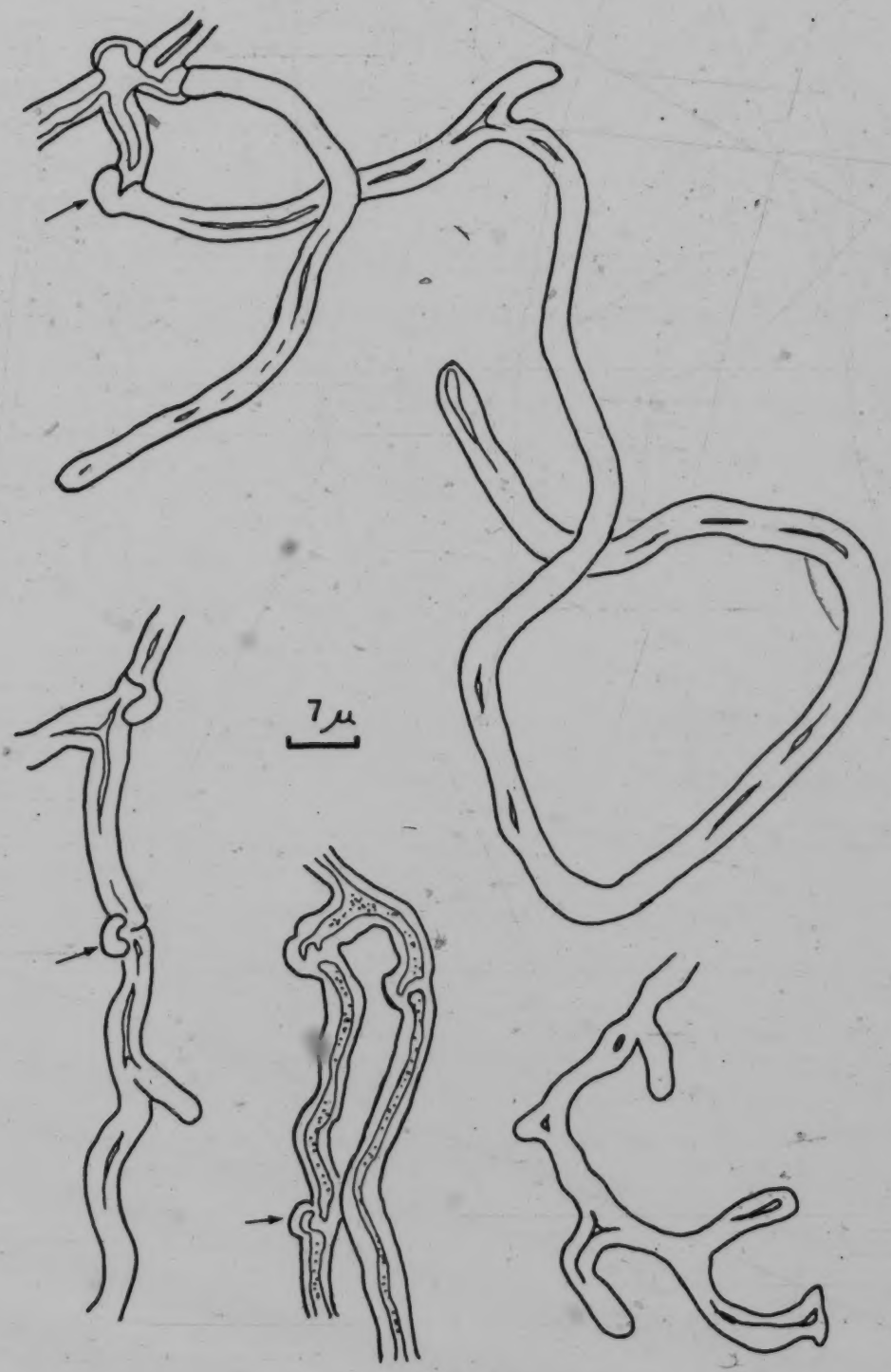
FIGURES 43-52. Microscopic structures in primordia and basidiocarps of *H. pargamenus*. Note that these components are representative for the three species.

FIGURE 43. Branched, multicellular, thin-walled generative hyphae.



43

FIGURE 44. Thick-walled, branched, multicellular generative hyphae. Note the thick-walled clamp connections (arrows).

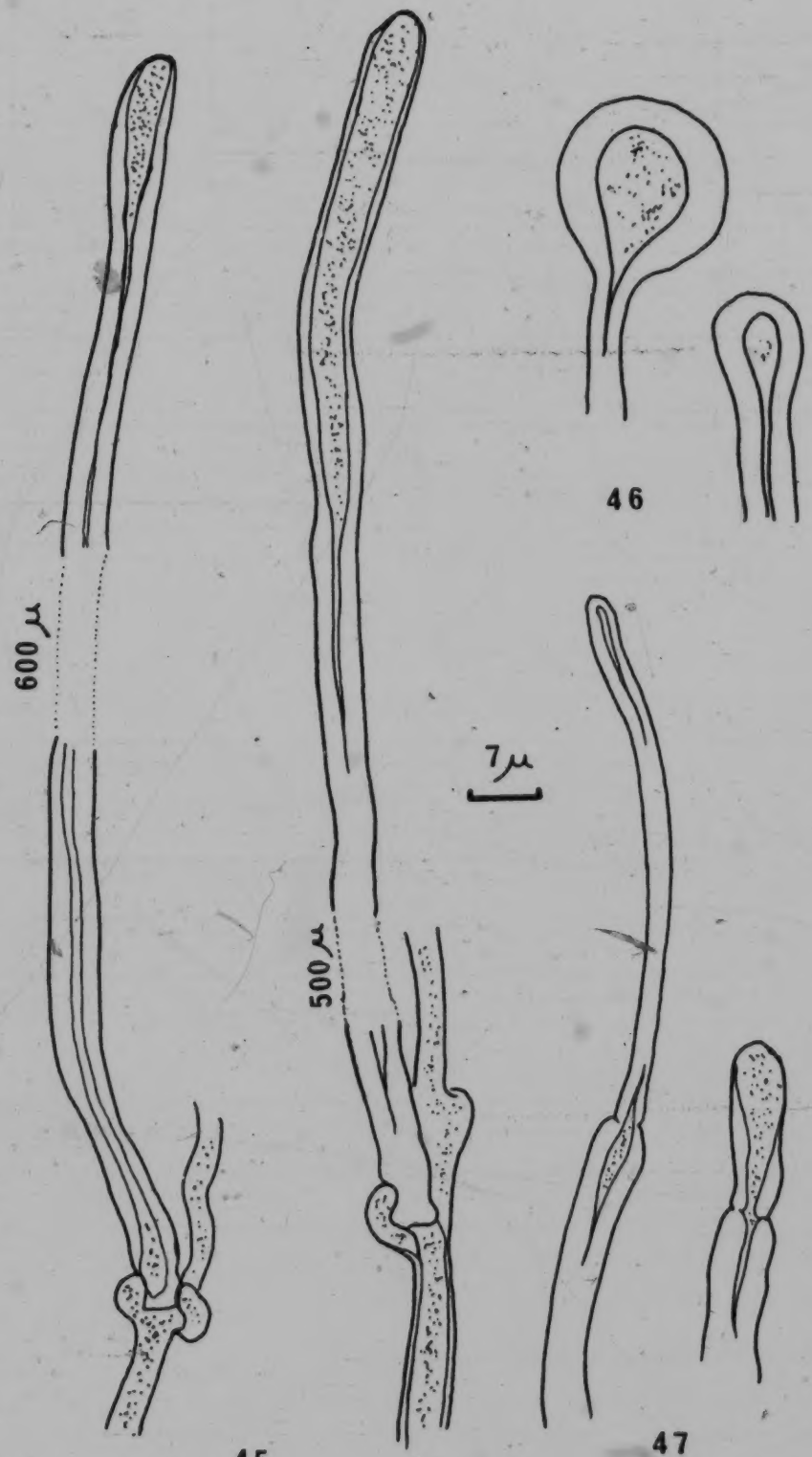


44

FIGURE 45. Long, unbranched, thick-walled, skeletal cells attached to thin-walled generative hyphae.

FIGURE 46. Swollen, thick-walled tips of skeletal cells.

FIGURE 47. Renewed growth at tip of skeletal cells.



45 46 47

FIGURE 48. Immature basidia.

FIGURE 49. Thin-walled, clavate basidia.

FIGURE 50. Curved-cylindrical basidiospores.

FIGURE 51. Thin-walled hymenial elements with narrow filamentous tips.

FIGURE 52. Capitate, crystal incrusted cystidia.

A - thick-walled

B - thin-walled

C - terminal position of cystidium.

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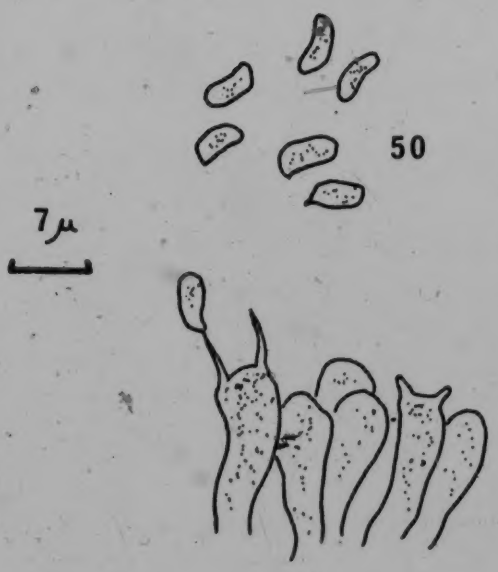


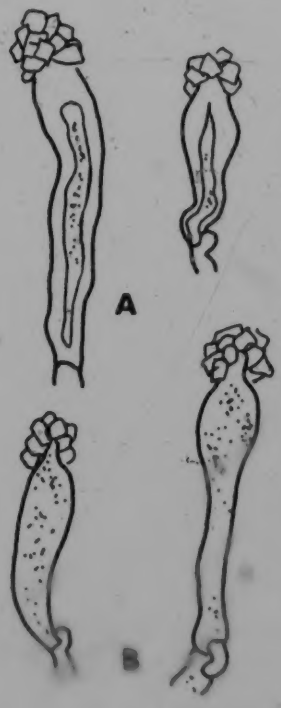
49

50

7μ

7μ

51



A

B

C

7μ

52

CHAPTER IV

CULTURAL STUDIES

Introduction

The complex basidiocarp produced in field environments has received most of the attention of early workers searching for characteristics by which polypores can be distinguished. Yet, the vegetative mycelium should not be ignored in taxonomic considerations. It is a very important part of the life-cycle of these fungi. Within the woody substrate, the saprophytic thallus, commonly a dikaryotic mycelium, persists for several years as it decays the log and periodically produces and nurtures the external reproductive structures. Because the mycelium in the wood is relatively undifferentiated, it has not provided distinguishing morphological features. Modern taxonomists, however, have begun to utilize the information obtained from studies of the non-reproductive (vegetative) mycelium in pure culture on artificial media. These studies are part of the biosystematic approach that I advocate in the study of polypore taxonomy. Under cultural conditions, the development and microstructure of hyphae is similar to that observed in differentiated non-hymenial tissues of natural basidiocarps. Comparative studies on standard media in a controlled environment facilitate the precise description of macro-morphology, micro-morphology and growth rate of colonies.

The work of Nobles (1948, 1965) provides standard procedures for the study of wood-decay basidiomycetes in culture. The results of her

comparative studies are presented in a key to 149 Hymenomycetes of which 114 are polypores (1965). Diagnostic characteristics in the key are: "production of extracellular oxidase, septation of hyphae, occurrence of special structures formed by the differentiation of hyphae, occurrence of conidia, chlamydospores and oidia, color of hyphae and mycelial mats, color changes in agar induced by growth of fungus, rate of growth, production of fruit-bodies, odor of cultures, host relationships and interfertility phenomena". Cultural features have been given systematic value (Nobles, 1958b, 1971) in the formation of groups which correspond to the segregations made by European mycologists using basidiocarp features as taxonomic criteria. Nobles (1971) recognized "close similarity" among 35 species of white-rot fungi (positive for extracellular oxidase tests) which produce cylindrical basidiospores and cultures containing "thin-walled, nodose-septate hyphae" and "fiber hyphae". She recognized "even closer similarity among cultures assigned...to *Hirschioporus*" which is characterized in culture within this larger group of polypores by conspicuous, short branches termed "incrusted, capitate cystidia" on the vegetative mycelium.

Nobles (1948, 1965) described cultures of *Polyporus* (*Hirschioporus*) *pargamenus* and *P. abietinus* on malt agar medium but found few microscopic features that distinguished the two species. Using the same procedures, Bakshi *et al.* (1969) confirmed her findings in their culture of these species in India. Other workers have described features of the vegetative mycelium of *H.* (*Polyporus*) *pargamenus* (Rhoads, 1918; Davidson *et al.*, 1942; Macrae, 1941) and *H.* (*Polyporus*) *abietinus* (Fritz, 1923; Macrae, 1941; Robak, 1942; Baxter, 1948; Cartwright and Findlay, 1958), but not necessarily using these standard procedures. Cultural studies of

H. subchartaceus, which closely resembles the other species in basidiocarp morphology are lacking.

In this chapter, development of the mycelium on sterilized wood blocks, Nobles' malt agar and various other media is described. The procedures for cultivation and description of colonies of the *Hirschioporus* species are those of Nobles (1965). The texture of the aerial mycelium is described using terms that she has adopted from initial cultural studies of polypores by Long and Harsch (1918). Unfortunately, Nobles has instituted a microstructural terminology for the mycelium in culture which is different from terminology coined by Corner for hyphae in basidiocarps. I have chosen to use the terms employed in the previous chapter to describe the hyphal components of the primordia and natural basidiocarps. For example, the fiber hyphae which Nobles (1965) defined as "hyphae with thick, refractive walls, hyaline or brown and lumina narrow or apparently lacking ... which usually arise as the elongated terminal cell of a hypha and are thus aseptate" are skeletal cells. The "thin-walled, nodose-septate (clamped) hyphae" are generative hyphae.

Nobles' quantification of growth rate is not very precise and does not distinguish cultures of *H. pargamenus* and *H. abietinus*, since both grow across a 9 cm petri-plate in 3-4 weeks. Bakshi *et al.* (1969) reported that *H.* (*Polyporus*) *abietinus* had a faster rate of growth than *H.* (*Polyporus*) *pargamenus* but did not provide the temperature at which measurements were made. They also reported that the optimum temperature for growth of *H. pargamenus* was 36°C, while the optimum for *H. abietinus* was 27°C. Other workers have given the optimum temperature for each species as 28°C (Davidson *et al.*, 1942; Baxter, 1948; Cartwright and Findlay, 1958; Humphrey and Siggers, 1933). These growth studies

are generally performed on one isolate of each species only. Cartwright and Findlay (1958) state that there may be a considerable difference between different strains of the same polypore species, an observation supported by growth rate studies of *P. abietinus* (Macrae, 1941). I have expressed the extension of colony margins of these three species as the average linear growth rate of four replicate colonies at 28°C, 18°C and 36°C in growth tubes containing Nobles' malt agar.

Fruiting strains of polypores are rare, and basidiocarps produced in cultures of *H. pargamenus* (Nobles, 1948) and *H. abietinus* (Cartwright and Findlay, 1958) on malt agar have been infrequently reported. Basidiocarp variability in different field environments has been a major problem in the identification and classification of *Hirschioporus* species based on these structures. The availability of fruiting strains provided an opportunity to compare basidiocarp development under standard conditions with development in the field. The development of basidiocarps in culture has not been described for *Hirschioporus* species yet study of this phase has provided important information concerning the homology of hyphae in the vegetative mycelium and in the basidiocarps. Furthermore, it has clarified the development and distinction of hyphal types observed in natural basidiocarps (Chapter III).

Materials and Methods

Dikaryotic and monokaryotic isolates, identified by the presence or absence of clamps, were used for cultural studies. The mycelium was isolated from small slivers of decayed wood under basidiocarps or from masses of germinated basidiospores collected on moist surfaces of 1.25% malt extract agar (Nobles' malt agar). Monokaryotic isolates were

obtained by picking single, germinated spores from agar surfaces. All isolates were grown at room temperature for two weeks and were stored on Nobles' malt agar at 4-5°C.

To study the growth of the mycelium on wood, blocks of sapwood from spruce and aspen were moistened at 30% of their dry weight, and were sterilized in the autoclave in closed containers. Sterile blocks were placed directly on the surface of colonies of the three fungi, 2 weeks old, growing in separate vessels containing Nobles' malt agar. Blocks 2 x 2 x 4 cm were cultured in deep petri-plates, 100 x 80 mm, while blocks 1 x 1 x 1.3 cm were cultured in glass tubes, 16 x 150 mm. All cultures were incubated in the dark, in plastic bags to maintain humid conditions, at 22°C for 3-6 months. Sections of soaked blocks were cut at 30 μ using a sliding microtome and stained with safranin and picro-aniline blue according to the procedure outlined by Cartwright and Findlay (1958) for detecting hyphae in decayed wood.

For growth of pure cultures on artificial media, the standard methods outlined by Nobles (1948, 1965) were employed. Dikaryon or monokaryon isolates were inoculated at one side of 9 cm petri-plates containing artificial media solidified with agar. The cultures were incubated in darkness at 25°C in a controlled environment chamber. Replicate colonies were examined at weekly intervals for six weeks. For microscopic observations, small pieces of mycelium from the advancing margin and older parts of the colony were squash-mounted in 5% KOH coloured with a drop of 1% aqueous phloxine. Nobles' 1.25% malt agar was used as well as 3% malt agar, 2% malt agar plus glucose and bactopeptone, aspen or pine sawdust plus glucose in a mineral medium solidified with agar, and a mineral agar medium plus glucose and thiamine (Appendix III).

Measurements of growth rate on Nobles' malt agar at 36°C were recorded for plate cultures, while growth rates at other temperatures were measured in growth tubes. Plastic plates (4 replicates for each isolate) containing 20 ml medium were inoculated at the side using a 5 mm disc of agar and mycelium cut from the margin of colonies, seven days old. After one week, the radius of colonies was measured at two day intervals by applying a plastic ruler to the underside of plates held up to the light. Similarly, the growth of cultures in tubes was measured at 2-3 day intervals using four replicates for each isolate. The growth tubes are 16 mm in diameter and 20 cm long bent up at each end at an angle of 45° (Fig. 56). Each tube contained 6 ml of medium making it approximately half-full. Colonies were incubated in darkness at constant temperature in controlled environment chambers. Isolates of each species were grown simultaneously at each temperature tested. The experiments at each temperature (18°C, 28°C and 36°C) were performed at different times during the period of the research. Also, results are presented as replicate averages in mm/day over a 9-18 day period.

Basidiocarps produced in culture on malt agar were examined microscopically by dissection in KOH and phloxine and by sectioning of fixed material embedded in paraffin as outlined in Chapter III. Sections were cut with a rotary microtome at 10-12 μ and were stained with safranin and fast-green (Jensen, 1962).

Observations

Morphology of the vegetative mycelium in wood

These *Hirschioporus* species show no substrate specificity in culture. The following description applies to all three species.

Within the wood, undifferentiated hyphae are found in the rays and in the lumen of vessels, tracheids and fibers. The hyphae which are thin-walled, narrow (1.5-2 μ in diameter) with clamps at septae, grow longitudinally and permeate the wood in all directions by frequent branching. The penetration of pits and thick walls results in bore-holes of the same diameter as hyphae or larger. Hyphae emerge from the transverse face of the block and form on the surface, mats of tangled, thick- and thin-walled generative hyphae (2-3 μ wide) and long, terminal, aseptate skeletal cells with thickened walls (2-5 μ in diameter). Regardless of substrate, the surface mat of *H. pargamenus* takes the form of small, cottony tufts or felted strands (Fig. 57), while that of *H. subchartaceus* is profusely cottony and raised (Fig. 57, right). The surface mat of *H. abietinus* is sparse, stranded (Fig. 58) and, in some isolates, barely detectable.

Morphology of vegetative mycelium on artificial substrates

Since microscopic and macroscopic features of the dikaryotic mycelium of *Hirschioporus* species are very similar, a description of the morphology and development of colonies and hyphae of *H. pargamenus* is presented. Distinctive characteristics of *H. abietinus* and *H. subchartaceus* mycelia are noted.

As in wood, the hyphae of *H. pargamenus* within the agar are thin-walled, 1.7-4.2 μ wide, with clamps. Hyphae of the submerged mycelium are much branched and contorted with irregular, vesiculate swellings (Fig. 65B). At the immediate surface of the agar, these thin-walled generative hyphae have regular cells (Fig. 65A). The colonies of *H. pargamenus* extend radially by the growth of thin-walled generative hyphae in the appressed margin. A continuous front of radial growth, 5-10 mm

wide is maintained by the growth of lateral branches into the advancing margin. After 10-14 days the generative hyphae give rise to thin-walled, short branches 3-5 μ wide, with slightly swollen tips incrusted with fine, hyaline crystals (Fig. 65C). These cystidia-like structures are observed with greater frequency as the colony ages. Segments of the thin-walled hyphae completely incrusted by fine granules are also observed in the older mycelium. Behind the advancing margin of *H. pargamenus* colonies, terminal cells of the generative hyphae grow and thicken toward their base to form long (200-1500 μ), unbranched aseptate structures termed skeletal cells (Fig. 67). In older portions of the colony, the walls and clamps of generative cells occupying an intercalary position become uniformly thickened. The lumen of these modified generative hyphae may be almost completely occluded (Fig. 66). Terminal and intercalary, thick-walled swellings (Fig. 68) are occasionally observed on older hyphae. The thin-walled and thick-walled, branching hyphae, and the skeletal cells are entangled to form the felted surface mat (aerial mycelium).

The white colonies of *H. pargamenus* have no odour and produce no change in the colour of the malt agar medium. The advancing margin gives a dark blue colouration when tested with a drop of brown, alcoholic gum guaiacum. This reaction is interpreted as a positive test for presence of extracellular oxidases. The presence of these enzymes was confirmed by the production of brown diffusion zones and lack of growth on gallic acid medium. Texture of the mycelial mats of *H. pargamenus* colonies varied from cottony-tufted to felty and stranded (Fig. 53). Cottony and felty are terms adopted from Nobles (1965). Cottony refers to abundant long hyphae, loosely tangled and spreading in all directions. Skeletal

cells are largely responsible for the raised cottony texture of aerial mycelium. Felty describes a more interwoven and matted aerial mycelium. The "strands" observed in the colonies are loose aggregates of branched, entwined hyphae growing in a parallel arrangement.

In terms of types of hyphae, the colonies of *H. pargamenus*, *H. abietinus* and *H. subchartaceus* are identical. Moreover, all three species in culture have white surface mats, lacking any odour and producing a blue colour when tested with gum guaiacum for extracellular oxidase. The texture of the surface mats of *H. subchartaceus* and *H. abietinus* differs from each other and from that of *H. pargamenus*. Colonies of *H. subchartaceus* after four weeks are distinctly cottony and raised due to an abundance of long, skeletal cells in the aerial mycelium, while they are less abundant in the surface mat of *H. abietinus* which is usually sparse, appressed and subfelty. However, a single isolate of *H. abietinus* (T48) developed a cottony mat containing many loosely entangled skeletal cells. After several transfers, the surface mat of this isolate became appressed and subfelty.

The microstructure of the mycelium of *Hirschioporus* species did not vary when different media were used. However, increased nutrients (sugars and vitamins) in these media promoted the production of dense surface mats compared to growth on Nobles' malt agar. This type of growth was accounted for by the abundance of skeletal cells. A violaceous pigmentation was noted in the colony margin of an isolate of *H. abietinus*, T48, after it had grown on mineral salts agar plus glucose and thiamine for 10 days. The pigment did not diffuse into the agar and was localized in a narrow band (2-3 mm wide) of compact, thin-walled hyphae containing yellowish, granular material.

Monokaryotic mycelia of each species developed the same colony morphology and hyphal types as the dikaryotic mycelia (Fig. 69). As in dikaryons, texture of the surface mats after 3-4 weeks was the only morphological distinction between the species.

Growth rates of dikaryotic mycelia

The growth rates of colonies of each species presented as the average daily increment of marginal extension, are shown in Table 3. Variation is evident between different isolates of the same species, particularly isolates of *H. abietinus*. Moreover, the rate of growth of the same isolates differed with time. T93 and T29, isolates of *H. abietinus*, were consistently slow growing. In a repetition (R_2) of an initial experiment at 28°C (R_1), the growth rate of certain isolates of *H. pargamenus* (T30) and *H. abietinus* (T587) was considerably reduced. Of the three temperatures tested, 28°C generally produced the greatest average growth rates. At 28°C, the rate of linear growth of *H. subchartaceus* isolates was greater than that of *H. pargamenus* or *H. abietinus*, whose isolates had similar growth rates (Table 3). At 36°C the growth rate of all isolates was much lower. *H. abietinus* isolates were sensitive to this high temperature and some showed no growth from the inoculum. *H. subchartaceus* isolates, on the other hand, were able to grow better than the other two species at 36°C (Table 3). Decreasing the temperature to 18°C resulted in a similar growth rate for isolates of *H. abietinus* and *H. subchartaceus*, but reduced rates for isolates of *H. pargamenus*.

Basidiocarps developed in culture

Because the growth and development of basidiocarps is basically the same for the three *Hirschioporus* species, a description of the process is provided for *H. pargamenus*, followed by an outline of the differences shown by *H. subchartaceus* and *H. abietinus*.

Most of the isolates of *H. pargamenus* (83%) produced basidiocarps after 5-8 weeks on thin malt agar surfaces, usually at the edge of inverted plates on the lab bench. Exposure to light was a necessary treatment for the stimulation of fruiting. Compact white to violaceous tufts of thin- and thick-walled, tangled hyphae appeared on the surface mats. These primordial structures grew by the extension of parallel, thin-walled apices of generative cells and skeletal cells in a peripheral, violaceous margin. In culture, only the hymenial surface of basidiocarps developed. From papillae (Fig. 59), violaceous, thin tooth-like pores developed by the uneven, vertical extension (3-4 mm) of walls (Fig. 60). Occasionally, tubes developed from primordial ridges to form radially elongate pores (Fig. 61). The growth and development of the basidiocarp in culture was identical to that of the tubes in natural basidiocarps. The growing edge of the tubes remained sterile and consisted of the thin-walled tips of skeletal and generative cells in a parallel arrangement. The long, skeletal cell was developed by means of growth and thickening, which began 10-40 μ behind the apex and proceeded to the base of a terminal, generative cell. Within the tube wall, some generative hyphae became thick-walled. Thin-walled, generative hyphae grew outwards and branched repeatedly to form the palisade-like hymenium consisting of terminal cells modified to form capitate, crystal-encrusted cystidia (Figs. 72, 73) and clavate basidia (Fig. 70). Thin-walled cells in the

hymenium and interwoven generative hyphae beneath it were filled with granular cytoplasm. Basidiospores of *H. pargamenus* (Fig. 71) were thin-walled, hyaline, curved cylindrical, 5.4-6.3 (-7.4) x 2.0 µ.

Fruiting was less frequently observed in isolates of *H. subchartaceus* (23%) and *H. abietinus* (27%). Basidiocarps developed in a similar manner, but there were macroscopic distinctions in the configuration of the hymenial surface similar to those of natural basidiocarps. For example, the hymenial surface of *H. subchartaceus* developed from primordial ridges with thicker tube walls (Fig. 63) resulting in a poroid configuration (Fig. 64). Within these walls there was a greater proportion of loosely interwoven thin-walled hyphae than in the other species. The hymenial surface of *H. abietinus* consisted of thin-walled tubes showing irregular growth and poroid configuration (Fig. 62), almost labyrinthiform in some basidiocarps. Within the hymenium of *H. abietinus* basidiocarps, thick-walled cystidia were more abundant than in either *H. pargamenus* or *H. subchartaceus*. As in natural fruiting-bodies, the basidiospores provided the only microscopic distinction. The basidiospores of *H. subchartaceus*, being 6.3-7.4 (-11) x 2.0-2.5 µ were larger than those of *H. pargamenus* and similar in size to those of *H. abietinus* which were 5.5-6.3 x 2.2 µ.

Discussion

The primary aim of cultural studies was to determine features by which *H. pargamenus*, *H. abietinus* and *H. subchartaceus* could be distinguished. Studies of the vegetative mycelium on natural and artificial substrates yielded few morphological distinctions. However, there were certain physiological differences in growth response at selected temperatures, and studies of basidiocarps produced in culture

supported distinctions reported for basidiocarps in nature.

The fundamental, undifferentiated, thin-walled hyphae of the vegetative mycelium growing in wood is not a source of morphological distinctions between different *Hirschioporus* species. Moreover, the morphology of this mycelium is no different from that reported for *Polyporus adustus* (Kennedy and Larcade, 1971), *Coriolus hirsutus* and *C. pubescens* (Edwards, 1972) or *Fomes cajanderi* (Wong, 1973). Consequently, I do not accept the general conclusion drawn by Proctor (1941) that it would be "entirely feasible" to identify wood-destroying fungi by their hyphal characteristics in decayed wood. A lack of distinguishing features was demonstrated also by Roff (1964) who made a study of pure cultures of a variety of fungi decaying selected coniferous wood, including *Polyporus abietinus*.

On the surface of wooden blocks in culture, the hyphae become differentiated into types identical to those observed in the surface mats on logs in the field. However, the morphology of thick- and thin-walled hyphae, themselves, is similar for the three species. The tendency of *H. subchartaceus* to produce extensive mycelium on the surface of logs in nature, is paralleled by the consistent and copious production of a raised, cottony mat on the surface of blocks in culture. The texture of the surface mat distinguishes *H. subchartaceus* from *H. pargamenus* and *H. abietinus* which produce felty to stranded and appressed to subfelty mats, respectively, on similar substrates. The factors that trigger the differentiation of thin-walled hyphae emerging from a relatively uniform microenvironment inside decaying blocks incubated at constant temperature, in the dark, and inside closed containers must be more subtle than the fluctuating conditions of temperature, light and

humidity imposed upon hyphae on the surface of logs in the field. Studies of the factors governing development of basidiocarps of *Polyporus adustus* (Larcade, 1970; Kennedy and Larcade, 1971), and *Gloeophyllum saepiarium* (States, 1972; States, 1969) in the field indicate that light, temperature and particularly humidity are important morphogenetic factors. Yet these studies quantify the external "microenvironment" around developing basidiocarps. More information is required concerning the temperature, aeration and water relations inside the wood compared to the external environment.

Microscopic examination of the types of hyphae in the vegetative mycelium growing on artificial substrates yields no qualitative, morphological distinctions between these species of *Hirschioporus*. Differentiation of hyphal types occurs on the agar surface regardless of nuclear constitution. Dikaryotic and monokaryotic mycelia, in other words, have the same types of hyphae except for clamps. The morphology of hyphae on various media is stable in culture. The same types of hyphae are found in the surface mats and basidiocarps produced in culture. Similarly, the hyphae in these basidiocarps are identical to those of natural basidiocarps. Since developmental morphology of hyphae growing in nature and culture is identical, I consider the undifferentiated mycelium in agar to be analogous to the mycelium inside wood, while the differentiated aerial mycelium in culture is comparable to the mat on the surface of logs. In natural and cultural environments, primordia on the surface mats grow and develop into sporulating basidiocarps. Therefore, studies of the mycelium in culture and in basidiocarps in nature are complementary, and I object to the categorical statement made by Donk (1971) that "cultural mycelial mats are mere ghosts of natural fruitbodies....if they are comparable at all". To emphasize the point, I have

chosen to employ Corner's terms which describe hyphae in natural basidiocarps for my description of hyphae in the vegetative mycelium and basidiocarps in culture.

The thin-walled, septate, branching hyphae constitute the fundamental, generative hyphae which give rise to the other forms. Within the agar, the generative hyphae are vesiculate and contorted compared to the regular cells of the surface hyphae and marginal mycelium, probably due to poor aeration within the agar. In the older mycelium, intercalary cells and short, lateral branches of generative hyphae develop thickened walls. But, thickening of preformed elements such as these must be distinguished from the formation of long, aseptate, terminal skeletal cells produced on the generative hyphae. In skeletal cells growth proceeds by the extension of the apex while walls thicken increasingly towards the base of the cell. Thickening of generative cells subtending the skeletal cells results in a long, terminal element with thickened clamp connections similar to the "fiber hyphae with clamps" reported for *Cerrena unicolor* (van der Westhuizen, 1963). But, the intercalary thickening of generative cells in *Hirschioporus* species is only found in older mycelia, as in natural basidiocarps. The short branches with slightly swollen tips incrusted with crystals resemble cystidia in the hymenium of natural basidiocarps. Their different shape may be accounted for by the different "morphogenetic field" (Donk, 1971) in which they are produced. Cystidia have not been observed in the mycelium on the surface or inside wood, although Zycha and Knopf (1966) reported seeing them as "glassy" elements with a hand lens on the mycelium in the wood. The function of these cystidia-like structures remains unclear. Their occurrence on rich, nutrient agars would suggest some physiological

function, perhaps excretory as was suggested for cystidia in the hymenium. A determination of the identity of the crystals and a manipulation of substances in the medium would be helpful in elucidating their role on the vegetative mycelium. The major significance of cystidia in these studies is the close relationship that they indicate between species in the genus, *Hirschioporus*. The occurrence of similar capitate-incrusted cystidia in the cultures of *Polyporus versatilis* (Bakshi *et al.*, 1969) suggests that this taxon should be classified in the genus, *Hirschioporus*. Macroscopic and microscopic similarities between the basidiocarps of these *Hirschioporus* species and *P. versatilis* (Overholts, 1953) support this argument. A similar study of cultures of *Polyporus sector* and *P. trichomallus* which have these cystidia in the hymenium of natural basidiocarps may indicate that the genus, *Hirschioporus*, should include them as well.

Texture of surface mats provides a general, macroscopic feature by which cultures of *Hirschioporus* species can be distinguished. This conclusion is supported by the observations of Macrae (1941) and Nobles (1948, 1965) who studied *Polyporus pargamenus* and *P. abietinus*. Nobles (1948, however, included isolates of *H. subchartaceus* in her study of *H. pargamenus*. For example, she listed isolates made from collections on *Populus* wood. Examination of the voucher specimens of at least one of these collections on *Populus*, DAOM 5216, revealed that it was *H. subchartaceus*. Such an inclusion would account for the use of the term, "cottony" to describe the aerial mycelium of *H. pargamenus*. The observations reported in this chapter show that aerial mats of *H. subchartaceus* were raised and cottony, whereas, the mats of *H. pargamenus* were tufted to felty and stranded. The similarity of these two surface

mats shows the close relationship of the species and makes differences of this feature difficult for the novice observer to detect. The texture of *H. abietinus* mats was subfelty and appressed. But, the observation of a cottony isolate of *H. abietinus*, T48, detracts from the significance of this character as an absolute distinction. It must be pointed out that similar textural differences in the surface mats were evident on the surface of wooden blocks.

All the colonies were white on artificial substrates, but a single isolate, again T48 (*H. abietinus*) exhibited a violaceous margin when grown on Faro's mineral medium plus glucose and thiamine. The localization of the pigment in compacted, thin-walled hyphae agrees with observations made of pigmented areas in natural basidiocarps and cultural basidiocarps. The production of pigment in vegetative mycelium by this strain has not been pursued further, but it provides a good source of material for chemical and physiological factors that govern pigment production in controlled environments. From these observations, one could perhaps conclude that any vegetative mycelium is capable of producing pigmentation given the right conditions, and that the compact association of thin-walled hyphae enhances its visibility. For pigment studies to have taxonomic value comparative analysis of the extracted substances from all three *Hirschioporus* species is required.

Nobles (1958, 1971) used the character, presence or absence of extracellular oxidase, as a major feature in splitting the polypores into two groups corresponding to white-rot fungi (positive for oxidase) and brown-rot fungi (negative for oxidase). Donk (1971) has raised an objection to the use of this criterion because 10% of species cannot be assigned to either group. However, in *Hirschioporus* cultures the presence

of oxidase is detected by the gum guaiacum test (Nobles, 1958a) and the gallic acid test (Davidson *et al.*, 1938) confirming the observation that these are white-rot organisms decaying lignin. Studies by Garren (1938a, 1938b) show that *P. abietinus* is capable of decaying isolated cellulose in culture as well. The activity of these enzymes can vary with the age of the mycelium, temperature and pH (Käärik, 1968) and these factors may contribute to the variation in response to indicator substrates in culture. The important feature is that these are white-rot fungi and that a general test may not be suitable for the enzymes in all fungi.

Snell *et al.* (1928) indicated that *H. pargamenus* and *H. abietinus* could be distinguished in rate of growth at certain temperatures. The studies reported here show that the response at 28°C, 18°C and 36°C is distinct for the three *Hirschioporus* species. Using several isolates, I have shown that there is considerable variability in linear growth rates between isolates of the same species. Bakshi *et al.* (1969) reported the maximum growth of *H. pargamenus* at 36°C, but I have found that colonies barely grew at that temperature. At 28°C, the temperature assumed to be optimal, the linear growth rate of *H. subchartaceus*, *H. pargamenus* and *H. abietinus* could be ranked as first, second and third, respectively. Previous workers (Humphrey and Siggers, 1933; Davidson *et al.*, 1942; Cartwright and Findlay, 1958) have tended to use single isolates in reports of linear growth rate for each species. But, I have found that measuring the growth rate of several isolates and reporting the range of response at a given temperature is a more precise representation for each species. Because of the variability between isolates and the problems of standardization of growth conditions and inoculum potential, the use of growth rate as a taxonomic criterion is not popular. Use of growth-

tubes to measure linear growth results in a better indication of relative rate of colony extension than the "time required (in weeks) to cover a 9 cm petri plate" (Nobles, 1965).

Although temperature requirements have not been fully investigated in this study, the results give an indication that there are temperature preferences for each species. For example, *H. subchartaceus* grows faster than the other two species at higher temperatures (36°C) while *H. pargamenus* grows slower at the lower temperature (18°C). Temperature preferences in culture are paralleled by preferences apparent in field habitats. For example, in the field, *H. subchartaceus* basidiocarps are frequently collected in open, sunny localities. Moreover, the general intolerance of *H. abietinus* to high temperatures in laboratory culture parallels the infrequent collection of this fungus from tropical regions. Further studies are required on the temperature preferences of these species in field and cultural situations. Temperature studies of *P. versatilis* and *P. sector* which Overholts (1953) regards as southern analogues of these species would be interesting.

Of the three species, isolates of *H. pargamenus* are distinctive in their tendency to fruit in culture. The study of basidiocarps developed in culture is important in their confirmation of microstructural and macrostructural basidiocarp differences observed in the field environment. Distinctive characteristics include size of spores and configuration of the hymenial surface. As in nature the basidiospores of *H. subchartaceus* are distinctly larger than those of *H. pargamenus*, and the hymenial configuration of this species is poroid while that of *H. pargamenus* is tooth-like. However, tooth-like structures have been noted in cultures of *H. subchartaceus*. Thus, the configuration of the hymenium, as in

nature, must be regarded with caution when utilized for taxonomic considerations.

In summary, cultural studies of the vegetative mycelium provide very few distinctive morphological features between *Hirschioporus* species. Their importance lies in the elucidation of the development and morphology of hyphal types less easily discerned in the natural basidiocarps, and in the support of the close relationship shown by use of basidiocarp features in nature. Nobles (1971) presents a similar opinion: "Meanwhile, in cultures of all species, including those whose fruit-bodies are so difficult to dissect and whose hyphal systems are difficult to determine, generative hyphae and hyphae modified to form the characteristic microstructures of the species may be observed with ease". The final distinction of species in culture requires the use of supportive information which Nobles obtains from studies of the basidiocarps in nature. Moreover, for the distinction of *Polyporus pargamenus* and *P. abietinus*, she used the observation that *P. pargamenus* generally was collected on deciduous substrates while *P. abietinus* was usually collected on coniferous trees as a diagnostic feature (Nobles, 1958, 1965). In the systematic treatments of the polypores (1971, 1958) Nobles employed spore characters of natural basidiocarps in the formation of groups. I have shown that for *Hirschioporus*, at least, these spore features are also stable in the cultural environment.

In Nobles' key for the identification of wood decay fungi the diagnostic character, "interfertility phenomena" is found. This phrase refers to the use of genetical information involving the results of pairing isolates in culture. In the following chapter the results of such tests with *Hirschioporus* species are outlined.

TABLE 3. Linear growth rates (mm/day) of dikaryotic isolates of *Hirschioporus* species

Species	Collection	Temperature 18°C	28°C (R_2)	28°C (R_1)	36°C*
H. pargamenus	T360	2.4	**		0.1
	T73	2.3	5.1	5.8	0.3
	T592	1.8	5.1		0.6
	T728	1.6	4.8		1.1
	T30	1.6	2.7	5.0	0.2
	T745	1.2			0.5
	Average	1.8	4.4	5.4	0.3
H. abietinus	T54	4.8	5.6	5.2	0.1
	T48	4.7	4.0	5.3	0.0
	T587	4.1	1.8	4.0	0.0
	T58	3.7	5.2	5.5	0.1
	T93	2.1	3.0		0.3
	T29	1.3	2.8	3.4	0.2
	Average	4.4	3.7	4.7	0.1
H. subchartaceus	T68	5.2	5.6	6.4	0.6
	T588	4.4	6.4	6.5	1.2
	T31	4.2	7.8	6.6	0.6
	T122	4.2	6.7	6.6	0.9
	T744	3.7			0.7
	T72	2.7	8.5	7.2	1.4
	T590		5.5	6.1	
	Average	4.4	6.6	6.6	0.9

*growth in plates

**isolate from this collection not used

FIGURE 53. Felt-like, reticulate-stranded, white surface mat on Nobles' malt agar after 4 weeks. *H. pargamenus*, T592. X0.8

FIGURE 54. Raised, cottony, white surface mat on Nobles' malt agar after 4 weeks. *H. subchartaceus*, T72. X0.8

FIGURE 55. Appressed to woolly surface mat on Nobles' malt agar after 4 weeks. *H. abietinus*, T54. X0.75

FIGURE 56. Colony surfaces on Nobles' malt agar in growth tubes after 2 weeks. *H. subchartaceus*, T588. X0.75

FIGURE 57. Cottony, tufted surface mats of *H. pargamenus* T73 (left) and *H. subchartaceus* (T68 (right) on *Populus* blocks in culture. X1.0

FIGURE 58. Sparse, appressed surface mats of *H. abietinus*, T93 on *Populus* blocks. X1.0

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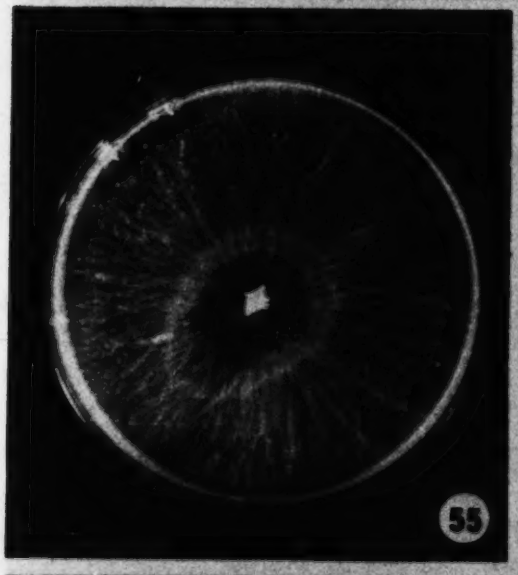
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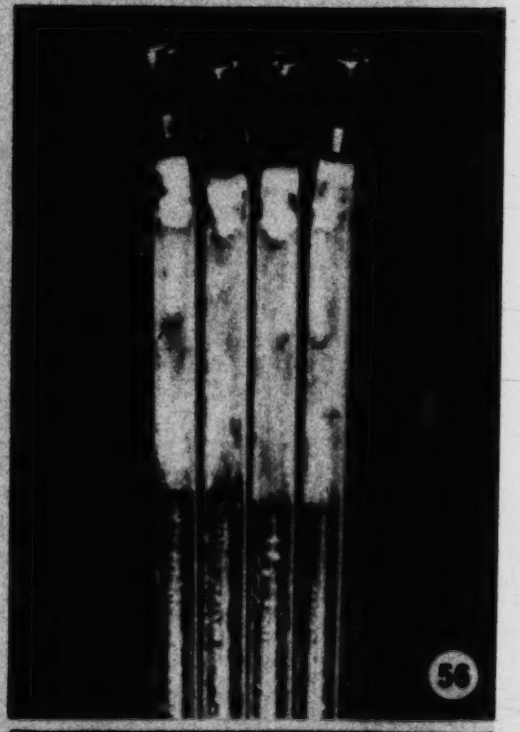
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FIGURES 59-64. Basidiocarps produced in culture.

FIGURE 59. Papillate primordia. *H. pargamenus*, T73. X10.0

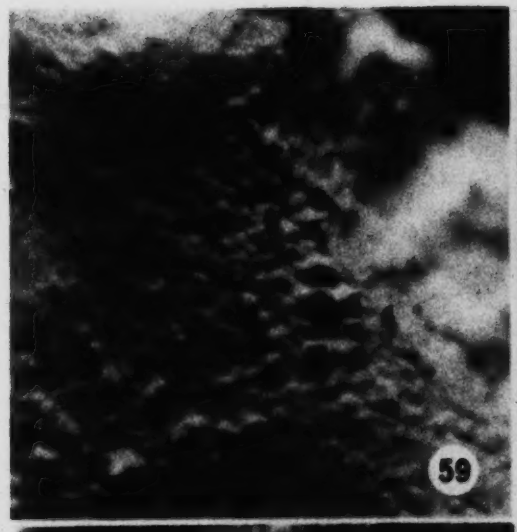
FIGURE 60. Tooth-like hymenial surface. *H. pargamenus*, T73. X10.0

FIGURE 61. Shallow, tooth-like ridges. *H. pargamenus*, T592, X10.0

FIGURE 62. Poroid to labyrinthiform configuration of the hymenial surface. *H. abietinus*, T92. X7.0

FIGURE 63. Ridged, tuft-like primordium. *H. subchartaceus*, T31. X10.0

FIGURE 64. Poroid hymenial configuration. *H. subchartaceus*, T114. X10.0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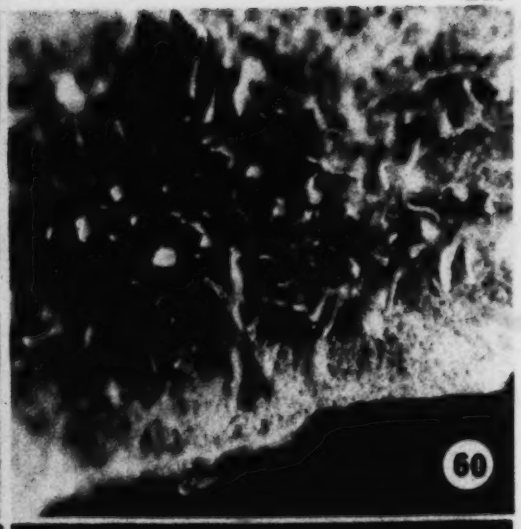
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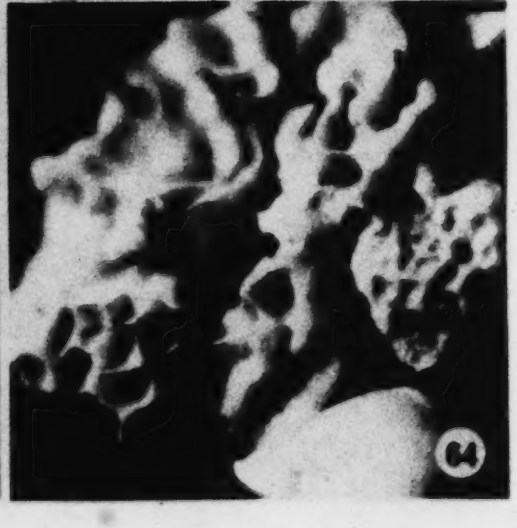
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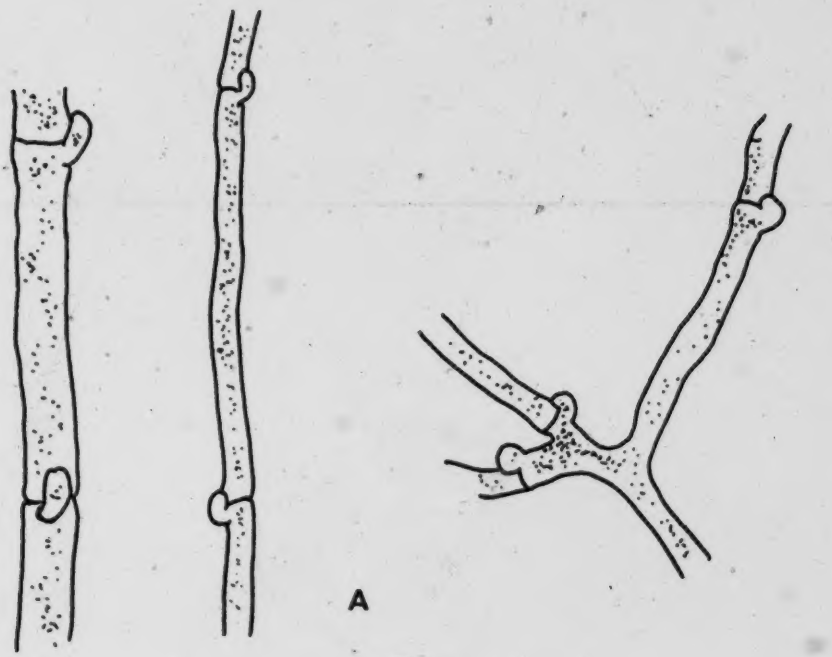
FIGURES 65-73. Microscopic components of vegetative mycelium and basidiocarps of *H. pargamenus* in culture. These components are characteristic of all three *Hirschioporus* species.

FIGURE 65. Thin-walled, multicellular, branched, generative hyphae.

A - regular generative cells

B - vesiculate, contorted hyphae submerged in the medium

C - capitate, crystal-incrusted cystidia-like structures on surface hyphae



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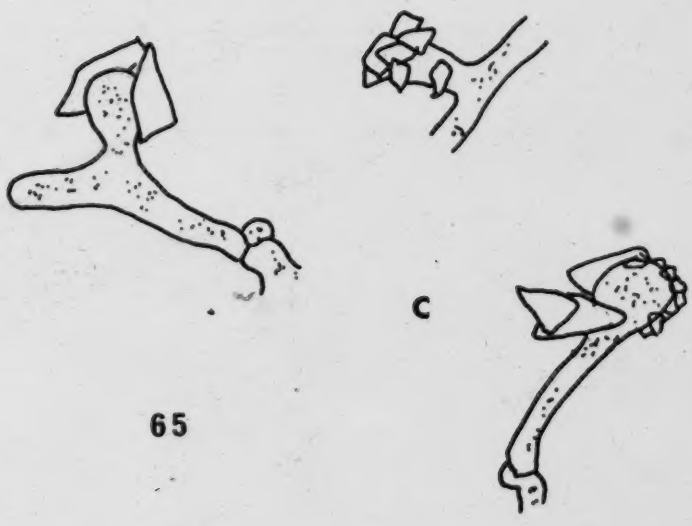

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FIGURE 66. Branched, multicellular, thick-walled, generative hyphae. Note thick-walled clamps (arrows) and the almost completely occluded cells.

7μ

FIGURE 67. Long, terminal, thick-walled skeletal cells attached to thin-walled generative hyphae.

FIGURE 68. Swollen, thick-walled tips of skeletal cells.

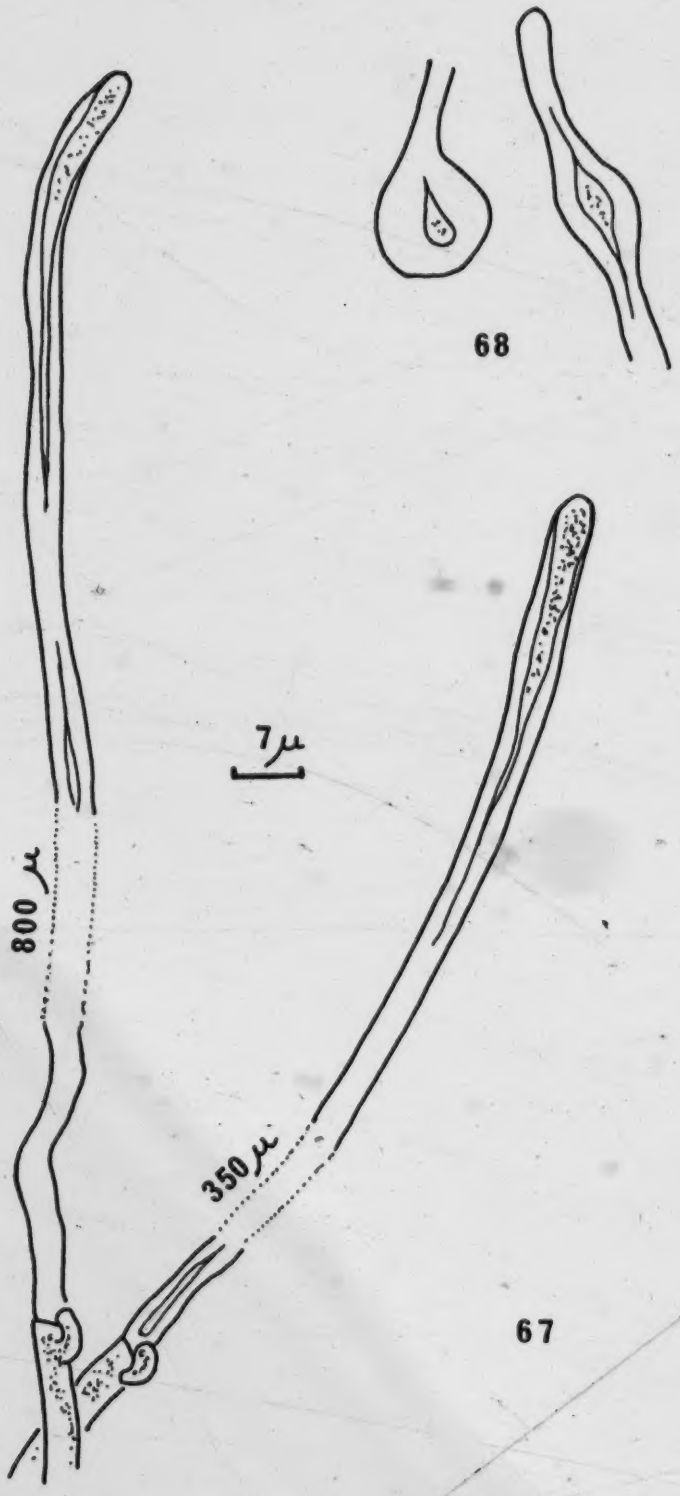

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FIGURE 69. Microstructural components of monokaryotic mycelium in culture.

A - skeletal cell

B, C - thick-walled generative hypha

D - thin-walled generative hypha

E - vesiculate, submerged hypha

F - capitate-incrusted cystidia-like structures on surface hyphae.

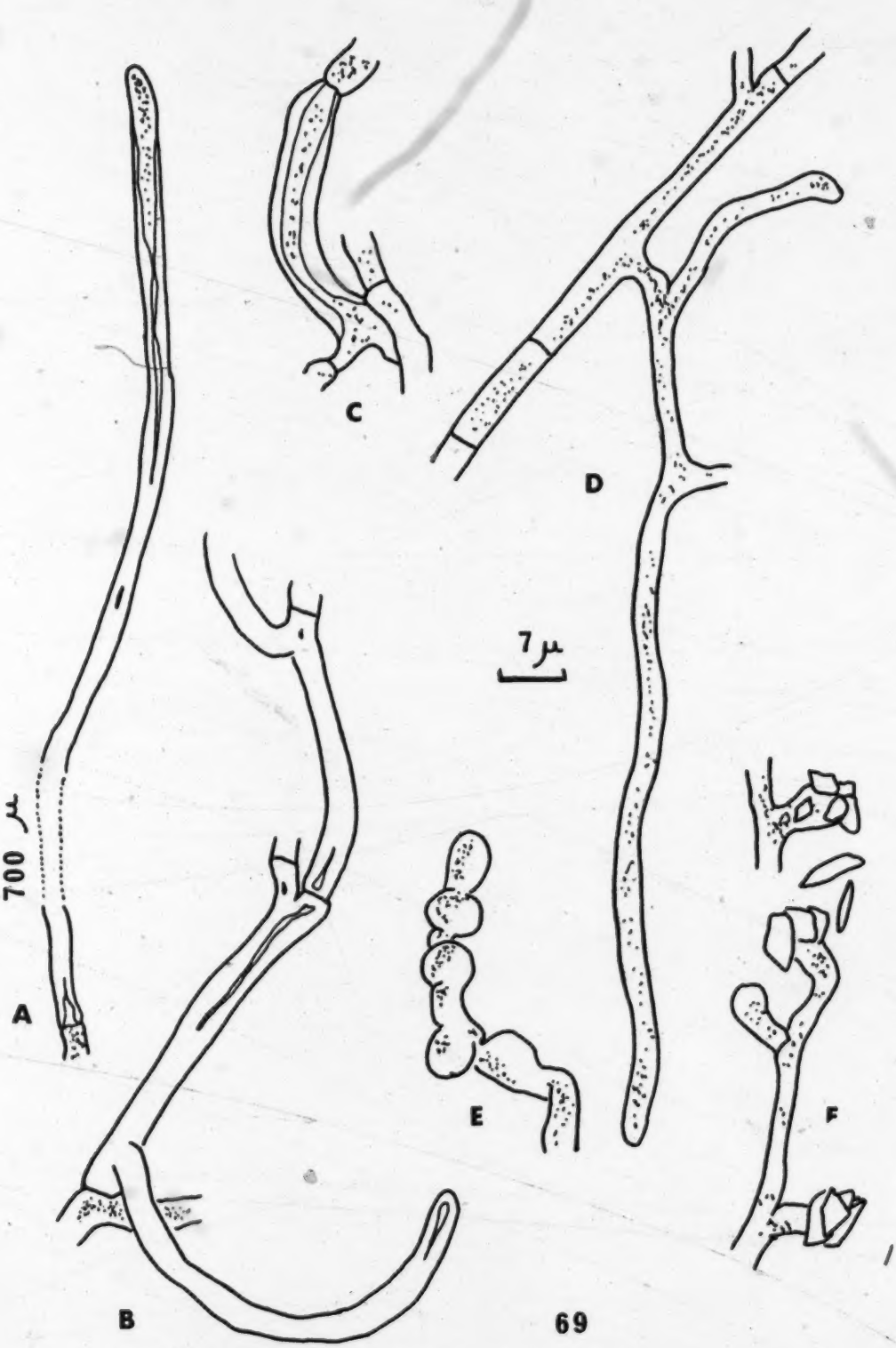

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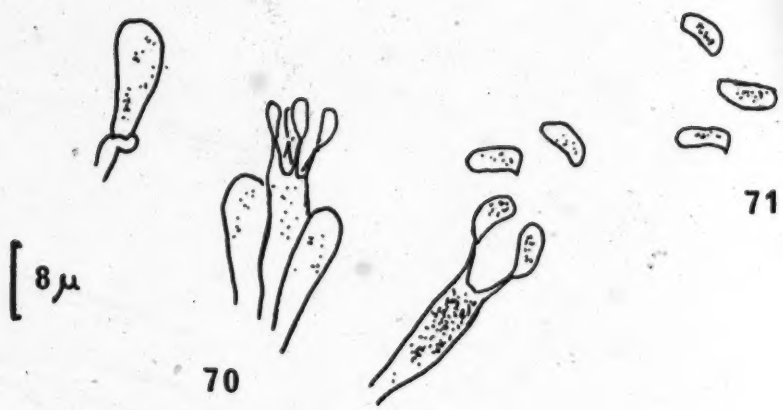
FIGURE 70. Clavate basidia in cultural basidiocarp.

FIGURE 71. Curved, cylindrical basidiospores.

FIGURE 72. Capitate-incrusted, thick-walled cystidia in hymenium.

FIGURE 73. Capitate-incrusted, thin-walled cystidia.



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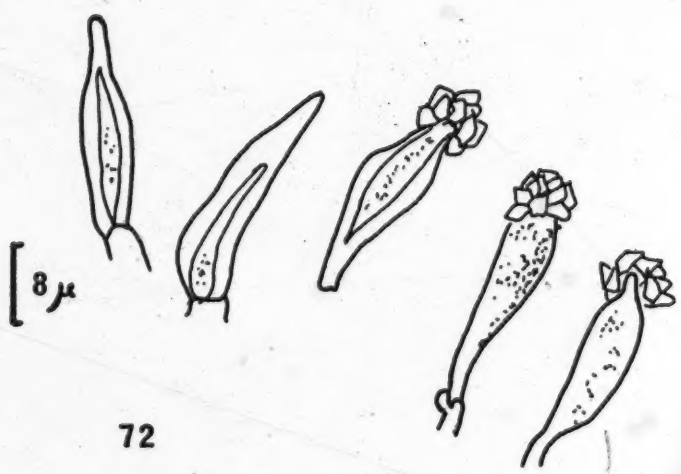

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CHAPTER V

GENETICAL STUDIES

Introduction

The genetical studies reported in this chapter deal with the sexuality of higher basidiomycetes and involve the fusion of undifferentiated cells in culture. Like most polypore species, these fungi are self-sterile and cross-fertile. This obligatory cross-mating, discovered independently by Bensaude and Kniep in the early part of this century (Whitehouse, 1949), is determined by an incompatibility mechanism involving one or two mating loci. Paired monokaryotic mycelia must have unlike alleles at these loci to be compatible and produce normal dikaryotic mycelium with normal clamp connections. Unifactorial or bipolar incompatibility is governed by one locus; bifactorial or tetrapolar incompatibility is governed by two loci, A and B (Raper, 1966). The alleles at these two loci segregate independently during meiosis to produce spores yielding from a single basidiocarp, monokaryotic mycelia of four mating types, A_1B_1, A_2B_2, A_1B_2, and A_2B_1. Cross-breeding is further enhanced by the occurrence of multiple alleles for these mating factors (loci) in different isolates of the same species. Multiple alleles were first discovered for *Schizophyllum commune* by Kniep and later for *Coprinus fimetarius* by Brunswik, as they attempted to explain the compatibility of isolates from different geographic locations (Raper, 1966). Whitehouse (1949) estimated the number of alleles for each locus to be of the order of 100. Fries and Jonasson (1941) reported 23 A-

alleles and 26 B-alleles in a sample of 16 basidiocarps of *H.* (*Polyporus*) *abietinus* collected from different localities in Sweden. Work by Papazian (1950) with *S. commune* has shown that the mating factors themselves are complex and consist of more than one locus. Intrafactor recombination may then be an explanation for the puzzling compatibility reported by early workers who attributed their anomalous results to mutation (Raper, 1966).

In her keys for the identification of wood-decay hymenomycetes, Nobles (1948, 1965) has incorporated the type of incompatibility, bipolar or tetrapolar, for each species where it is known. But this is not a distinguishing feature for the *Hirschioporus* species considered in this thesis because reports by Macrae (1941, 1967) and Nobles *et al.* (1957) indicate that all three taxa have a tetrapolar incompatibility mechanism.

Intersterility of monokaryon isolates, originally proposed by Vandendries as a criterion for delimiting species (Lange, 1952), has gained increased acceptance through its use by investigators who have cultured polypores on artificial media. The following is a quotation from Nobles and Frew (1962): "In practice, four (rarely three) single-spore cultures from each of two isolates to be tested are grown together in all possible combinations and mycelium from the line of meeting in each pairing is examined for the presence of hyphae with clamp connections. The formation of dikaryotic hyphae with abundant clamp connections in all pairings provides strong evidence that the two isolates belong to the same species, the failure to produce such dikaryotic hyphae indicates that they belong to different species". This criterion for the conspecificity of taxa is based on the production of clamps and thus

applies only to fungi bearing these structures on their hyphae in culture. However, some caution must be exercised in the use of these tests because hyphal fusions and the production of clamps is subject not only to the controls of genetical and physiological factors inherent in the mycelia (Raper, 1966) but to the influence of aeration (David, 1968) and chemicals in the medium (Ahmad and Miles, 1970). David (1968) showed that in submerged mycelium of *Gloeophyllum* spp. in liquid culture, clamps degenerated and failed to form due to these anaerobic conditions. Work by Ahmad and Miles (1970) with *S. commune* showed that concentration of sugars and arsenates in the medium could affect hyphal fusion and clamp formation. Similar effects have been reported by Butler (1968) for *Coprinus disseminatus* and by Kerruish and Da Costa (1963) for *Lenzites trabea*. In fact, the production of monokaryons in basidiomycetes by the action of toxic chemicals on dikaryotic mycelia is an accepted technique (Osborne, 1970) for isolation of haploids.

Reports of cases where clamp connections have been found at the junction or on one side of pairings between monokaryons of taxa identified as different species by morphological criteria, raise the question as to whether these are interspecific hybrids. Nobles and Frew (1962) recognized these rare clamps as an indication of close relationship between *Pycnoporus coccineus* and its close relatives, *P. sanguineus* and *P. cinnabarinus*. They did not regard them as very significant in the overall pattern of species intersterility. However, the interpretation of partial fertility between the taxa, *Peniophora duplex* and *Stereum pini* (European) by Weresub and Gibson (1960) reflected less courage in accepting a general pattern of intersterility. As a result, they treated the two taxa as subspecies of *Peniophora pini*, in spite of morphological

differences between their natural fructifications and regardless of the observation that the "hybrids" lost their ability to produce clamps in subsequent transfers.

Nobles and Frew (1962) demonstrated by two methods that the taxa whose single-spore isolates revealed "some sporadic formation of hyphae with clamp connections" when paired with *P. sanguineus* were actually *P. coccineus*: first, monokaryon isolates of these fungi were completely compatible with isolates of *P. coccineus*; second, dikaryon isolates of these taxa were able to "diploidize" monokaryotic isolates of *P. coccineus* but not isolates of *P. sanguineus*. The latter reaction is termed the Buller Phenomenon and has been used commonly to establish the identity of unknown dikaryotic mycelia in culture. Buller (1931) studied the dikaryotization of monokaryotic colonies of *Coprinus lagopus* after they had been inoculated with a small piece of dikaryotic mycelium. He discovered that dikaryotization would take place in non-compatible, illegitimate matings (*e.g.* $A_1B_1 + A_2B_2$ mated with A_1B_2) and hemi-compatible, legitimate matings (*e.g.* $A_1B_1 + A_2B_2$ mated with A_2B_2). The phenomenon was named after him, but investigations have revealed it to be a complex, incompletely understood process (Raper, 1966). Although the phenomenon has been utilized for pairings between species, the detailed investigations of it have been based on the study of pairings between monokaryons and dikaryons of the same species, notably *S. commune*.

The ideal situation is one in which the intersterility of taxa is related to morphological distinction, substrate preference or some geographic factor which provides a barrier to interbreeding. Many examples could be cited as illustrative of such a situation. Nobles and

Frew (1962) found macro- and micro-morphological and distributional differences throughout the world, that correlated with genetical distinction between three *Pycnoporus* species. Recently, Anderson *et al.* (1973) reported that *Pleurotus ostreatus* with its cream-coloured spore-print, slightly larger spores and growth rate faster than that of *Pleurotus sapidus* was found only on *Populus tremuloides*, while *Pleurotus sapidus* had a lilac spore-print and was found on a wide range of "angiosperm trees". These differences corresponded to the intersterility of the two "macroscopically indistinguishable" taxa which were not confronted by any geographical barrier to interbreeding. Ullrich (1973) reported intersterile groups in the *Sistotrema brinkmanii* aggregate that were correlated with distinctions in culture, but not geographical or substratal barriers. But there are also reports of intersterile groups which have no morphological distinctions, nor can they be related to any disjunction in geographic distribution or host preference. For example, Mounce and Macrae (1938) reported two morphologically similar, intersterile groups of *Fomes pinicola* in North America. Similarly, Lange (1952) reported three intersterile but morphologically indistinguishable groups in the taxon, *Coprinus subimpatiens*. However, most noteworthy in genetical studies in the genus, *Hirschioporus*, is the report of Macrae (1941, 1967) that two morphologically identical but intersterile groups of *H. abietinus* exist in North America without any relation to geographic distribution or substrate preference.

A number of genetical studies have been performed on *H. abietinus*. Macrae (1967) observed that the intersterility of *H. abietinus*, *H. fusco-violaceous* and *H. laricinus* was associated with distinctions in the hymenial configuration of the basidiocarps of the three, closely related

taxa. Previous to these studies, she demonstrated a definite intersterility between *Polyporus* (*Hirschioporus*) *abietinus* and *P. pargamenus* (the poroid and hydnoid forms), but did not conclusively distinguish the hydnoid form of *P. pargamenus* from the poroid form (*H. subchartaceus*) using this feature as a criterion. Moreover, there was not complete interfertility between these forms of *P. pargamenus* whose identity on the basis of morphology of the basidiocarp had been questioned by Rhoads (1918).

The genetical distinction between *H. abietinus* and the other taxa was accepted on the basis of the results of Macrae (1941) who paired several monokaryotic isolates and reported complete intersterility. However, because of the morphological similarities in basidiocarps and cultures of *H. pargamenus* and *H. subchartaceus* and because Macrae (1941) was unable to resolve a definite genetical distinction in her studies which showed an isolate of the hydnoid form of *P. pargamenus* to be partially compatible, using the clamp criterion with monokaryons of the poroid form, I have chosen to perform further monokaryotic pairings of these two taxa. Certain anomalies in the behaviour of monokaryotic isolates prompted the use of the Buller Phenomenon to substantiate the results of monokaryon pairings and to establish the identity of selected collections of *H. abietinus* using genetical tests as well.

Materials and Methods

For intracollection pairings, single-spore isolates (12-15) were obtained from fresh basidiocarps of *H. pargamenus* and *H. subchartaceus* previously identified by morphological criteria (Fig. 90). Single, germinated spores were picked from prints collected on the moist agar

surface (Nobles' malt agar) from pieces of basidiocarps glued to the lid of the petri-dish. These sporelings were transferred to culture tubes containing NMA and grown for two weeks at room temperature on the lab bench. During this time, the colonies were examined for the presence of clamp connections on hyphae. Monokaryotic isolates within each collection of *Hirschioporus pargamenus* (T728, T592) and *H. subchartaceus* (T72, T590) were paired in all combinations by inoculating the sides of plastic petri-plates containing 20 ml of NMA with 5 mm discs of agar and mycelium taken from the margin of 10-day-old cultures. The purpose of these intracollection pairings was to determine the incompatibility mechanism for each species and to identify the mating types of isolates in each collection. Compatible pairs of isolates were identified by intra-collection pairings of two isolates with other isolates from the same basidiocarp (Eggertson, 1953). Isolates of *H. pargamenus* collections, T360, T73, T745 and *H. subchartaceus* collections, T589, T122, T744 were paired in this manner.

In order to determine the presence of identical alleles in different collections of the same species and the interfertility or intersterility of isolates in different collections of the same or different species, respectively, a series of intercollection matings were performed. Ten isolates from each of T72, T590, T728, and T592 were paired in all combinations in plastic plates. Four isolates (two compatible pairs) were selected from each of the total ten collections of the two species for intercollection pairings in culture tubes. All pairings were made on Nobles' malt agar, and incubated at 25°C in controlled temperature chambers. After 2-4 weeks, mycelium taken from the contact zone and 2 cm on either side of it was mounted in 5% KOH and stained with 1%

aqueous phloxine, and examined for clamp connections (Macrae, 1967).

In the investigation of the anomalous behaviour of paired monokaryons which displayed a unilateral pattern of dikaryotization the Buller Phenomenon was utilized. Isolates of known mating type from the collection, T360, of *H. pargamenus* were mated to form "heterokaryons" with A-factors in common. This common-A heterokaryon was grown for ten days on NMA and was then paired with monokaryotic isolates of T360 with the four different mating types (A_1B_1, A_2B_2, A_1B_2, A_2B_1) and with monokaryotic isolates of *H. pargamenus*, T728, T592 and *H. subchartaceus*, T590, T589, T122, T72. After 4 weeks growth at 25°C as above, mycelium at the contact zone and on either side was examined for clamp connections.

Monokaryon isolates of *H. abietinus* obtained as previously described, from collections, T435, T587, and T586 as well as monokaryons of *H. pargamenus* and *H. subchartaceus* were paired with dikaryons of *H. pargamenus*, T360, T745, *H. subchartaceus*, T122 and *H. abietinus*, T48 on NMA. After 2-4 weeks, interactions in the form of demarcation lines were observed and mycelium in the contact zone and "monokaryon" side of the mating were examined for clamps.

A list of the collections with their geographic locations and substrates is provided in Table 4.

Observations

Intracollection pairings of *H. subchartaceus* and *H. pargamenus*

The results of intracollection pairings are presented in Figures 74-78. Both *H. pargamenus* (T728, T592) and *H. subchartaceus* (T72, T590) have tetrapolar incompatibility mechanisms. Within the isolates for each collection, the mating types could be recognized microscopically and

macroscopically on the basis of mycelial interaction. Using the convention outlined by Raper (1966), each monokaryon was assigned a mating type such as A_1B_1, A_2B_2, A_1B_2, A_2B_1.

Completely compatible pairings, namely, those with unlike alleles for both A- and B- loci, were recognized by the absence of any demarcation line at the junction of colonies, although sometimes there was less dense mycelial growth in this region. Complete intermingling of hyphae resulted in the production on both sides of the contact zone of dikaryon cells bearing normal clamps. Incompatible pairings (with both A- and B- loci alike, or with common A-loci) resulted in a gap of sparse mycelial growth at the junction of the colonies. At each side of this junction or bridging it, a raised line of aerial mycelium was sometimes observed. Clamps were not seen on the mycelium which was frequently vesiculate and gnarled. Blobs of extruded protoplasm stained with phloxine were occasionally observed at the tips of hyphae or occupying an intercalary position. Common-B pairings were readily detected. At the junction of the colonies there was a narrow (1-2 mm) gap of sparse mycelial growth bounded on either side by raised, tangled aerial hyphae. Within this double line, the cells were uninucleate to trinucleate with the hyphae bearing distinct "hooks" which stained darkly with phloxine and did not fuse with parent hyphae. Such hooked cells have been termed pseudoclamps by other workers (Raper, 1966).

After the partners of compatible pairings were arbitrarily designated as A_1B_1 and A_2B_2, the other mating types within the isolates of a single collection were assigned according to the common-B reaction and the process of elimination.

The rare occurrence of clamp connections and dikaryon cells on only

one side of compatible pairings was associated with slow-growing, appressed, dense colonies which acted as donors of nuclei. The mycelium of such colonies participating in the unilateral dikaryotization was characterized by gnarled hyphae bearing many short, contorted branches.

Fruiting was observed in compatible pairings of *H. pargamenus* but not of *H. subchartaceus*.

Intercollection pairings of *H. subchartaceus* and *H. pargamenus*

The results of pairing isolates from different collections of the same species showed that these collections of *H. subchartaceus* (Figs. 80, 86) and *H. pargamenus* (Figs. 79, 85) were completely interfertile and that there were no identical alleles. Unilateral dikaryotization (Figs. 80, 85, 86) in compatible pairings was observed with increased frequency (15% of *H. pargamenus* and 40% of *H. subchartaceus* pairings) compared to the intracollection pairings performed six months earlier (2% of *H. pargamenus* and 24% of *H. subchartaceus* pairings). Again, this phenomenon was associated with morphologically aberrant isolates which were slow-growing, appressed and served as nuclear donors while remaining monokaryotic and clampless themselves.

Monokaryotic hyphae intermingled freely and their fusion resulted in the formation of dikaryotic hyphae throughout both sides of pairings between isolates of collections of the same species. The surface mat in *H. pargamenus* pairings became white, felt-like and reticulate-stranded (Fig. 94), while the surface mat in *H. subchartaceus* pairings became white, raised and cottony (Fig. 93). The production of fruiting-bodies after 4-6 weeks was common in intercollection pairings of *H. pargamenus* collections but rare in *H. subchartaceus* pairings.

Isolates from different species were almost completely intersterile

(Figs. 81-84, 87). Rare, normal clamps and pseudoclamps were found to be unilaterally distributed (Figs. 82, 83, 87) or at the junction only of some interspecies pairings (Figs. 84, 87). The consistent production of clamps and dikaryon cells in 93% of pairings between T745 (*H. pargamenus*) and isolates from collections of *H. subchartaceus* (Fig. 87) was unexpected. The clamps were usually formed only at the junction of colonies, but occasionally they were unilateral on either the *H. subchartaceus* or *H. pargamenus* side of the pairing. Transfers from the junction of 16 of these "hybrid" pairings were slow-growing on NMA, appressed and abnormally plumose in branching pattern (Fig. 96) compared to normal dikaryons of *H. pargamenus* and *H. subchartaceus*. After 4-6 weeks normal dikaryons of *H. pargamenus* had fruited while the less vigorous "hybrids" developed many monokaryotic cells and pseudoclamps.

In addition to the general intersterility of the two species, white lines of demarcation were observed at the junction of interspecies pairings (Fig. 95). These lines were tough in texture due to profusely branched, entangled, thin-walled and submerged hyphae. Raised, cottony mycelium is only noted on the surface of these lines in older pairings where colonies attempt to grow over each other. Within the interaction zone of these interspecies pairings conspicuous, sickle-shaped, slightly swollen branches (Fig. 91) closely appressed to contacted hyphae were observed. No penetration or alteration to the contacted hyphae was seen. After some time, both the clasping branch and the contacted hyphae were observed to be devoid of cell contents. Both *H. pargamenus* and *H. subchartaceus* produce these branches in interspecies pairings. However, these structures were observed on rare occasions (less than 1%) in unilateral dikaryotizations involving abnormal isolates of intraspecies

pairings.

Use of the Buller Phenomenon

Mycelium transferred from the junction of pairings of isolates of *H. pargamenus* having common A-loci (factors) yielded slow-growing, appressed colonies containing hyphae with uninucleate and occasionally multinucleate cells lacking clamp connections. These heterokaryons, containing nuclei with mating types A_1B_1 and A_1B_2, were surrounded by the colonies of monokaryons paired with them (Fig. 92). Pairings of the common-A heterokaryon, T360-18 x T360-4, with non-compatible monokaryons of T360 did not result in clamp formation, while pairing with hemi-compatible monokaryons (A_2B_1 or A_2B_2) resulted in the unilateral production of clamps with the heterokaryon acting as donor of nuclei (Fig. 88). Similarly, these heterokaryons dikaryotized monokaryotic isolates of other collections of *H. pargamenus* (T728, T592) in a unilateral manner, but did not dikaryotize isolates of *H. subchartaceus* (Fig. 88).

Dikaryon isolates of T745 and T360 dikaryotized only monokaryotic isolates of various collections of *H. pargamenus* (Fig. 89), while T122 dikaryotized isolates of *H. subchartaceus* and T48 dikaryotized isolates of *H. abietinus*. These results were expected on the basis of morphological observations made in other studies and results of pairing monokaryotic mycelia. Hyphae of the same species intermingled freely so that dikaryon cells and clamp connections were observed throughout the colonies on both sides of the interaction zone. The failure of T48 to dikaryotize one monokaryotic isolate of *H. abietinus* was associated with the abnormal colony morphology of that monokaryotic isolate, T435-12. Interspecies confrontations of monokaryotic and dikaryotic mycelia were

marked by the formation of white, submerged lines of demarcation similar to those described for interspecies pairings of monokaryotic isolates. Moreover, in the interaction zone of interspecies "di-mon" (Raper, 1966) pairings, abundant clasping branches were observed for all three species. It must be pointed out, however, that these structures were also observed in intraspecies confrontations in which the monokaryotic partner was less vigorous, or abnormal in morphology such as observed for T435-12 (*H. abietinus*) x T48 and T590-16 x T122 (*H. subchartaceus*). Clasping branches were observed consistently between T745 and monokaryotic isolates of other collections of *H. pargamenus*.

Discussion

The results of intracollection pairings of monokaryons have confirmed the reports of heterothallic, tetrapolar incompatibility reported for *Polyporus* (*Hirschioporus*) *pargamenus* (Macrae, 1941; Bakshi and Chouhdury, 1961). The report by Macrae includes findings for the poroid form of *P. pargamenus* which is equivalent to *H. subchartaceus*. Although only three mating types were recovered from one collection of *H. subchartaceus*, T590, a larger sample would probably have revealed the fourth. The mating type of each monokaryon was deduced using standard procedures based on the observation of interactions of paired monokaryotic mycelia. For example, compatible monokaryons containing unlike A and B loci intermingled to produce dikaryon cells bearing normal clamp connections which fused with the parent hypha. Monokaryons with unlike B-loci were readily recognized by the production of double lines in the interaction zone which contained uninucleate and multinucleate cells with small pseudoclamps not fusing with the parent hypha.

The double line is termed a barrage (Raper, 1966). This aversion reaction of paired mycelia was originally described by Vandendries and Brodie (1933) and later by Brodie (1936) who applied the name, barrage, to the gap of sparse mycelial growth between monokaryons. My report of a barrage in *Hirschioporus* pairings confirms similar observations by Macrae (1941, 1967; Robak, 1942; Fries and Jonasson, 1941). The original use of the term, barrage, is somewhat confusing since loci we recognize as B-loci were designated as A-loci. In this chapter barrage is reserved for common-B pairings. The cause of this mutual aversion is not known, although by-products of metabolism of the two mycelia are implicated in the opinion of Vandendries and Brodie (1933). Studies with these *Hirschioporus* species indicate that aversion occurs in pairings other than common-B. Common-A and common-AB pairings are sometimes characterized by a dense line of aerial mycelium or a broad zone of diminished mycelial growth between the colonies. Even the compatible reactions display a less dense growth in the interaction zone which is soon bridged by intermingling hyphae.

Common-A mycelia immediately between the paired colonies contain gnarled, contorted hyphae with uninucleate and rare multinucleate cells on which blobs of cytoplasmic material have been observed. Similar observations have been reported for common-A heterokaryons of *S. commune* (Raper, 1966). In any case, it has been demonstrated that compatible pairings are not the only heterokaryotic products of hyphal fusion in *H. pargamenus* and *H. subchartaceus*.

The type of incompatibility mechanism or "interfertility phenomena" as Nobles (1965) terms it, does not serve to distinguish *H. pargamenus* and *H. subchartaceus*. Like *H. abietinus* and many other white-rot

hymenomycetes, these fungi have a tetrapolar incompatibility pattern (Macrae, 1941, 1967; Nobles, 1965; Nobles *et al.*, 1957). However, a knowledge of interfertility phenomena is important to an understanding of the results of intercollection pairings. For example, the presence of identical alleles in different collections of the same species results in less than complete interfertility of pairings (Whitehouse, 1949; Lange, 1952; Raper, 1966). If only a limited number of monokaryotic isolates were available for intercollection pairings, intersterility between the isolates might be interpreted as an indication that different species were involved, assuming that the mating types of isolates were not known. Consequently, knowing that the taxon is tetrapolar and heterothallic the researcher can select a suitable number of isolates (at least 10) for his investigation that will assure him that he is dealing with several mating types (Edwards, 1972) without actually knowing the mating types in every isolate. Should one select randomly only three or four monokaryotic isolates for each collection, he would possibly be faced with pairing results which would be misleading. The suggestion of this number of isolates by Nobles and Frew (1962) is based on the assumption that collections from different localities have unlike alleles.

Identical alleles have not been observed in my studies of *H. subchartaceus* and *H. pargamenus*. However, Macrae (1941) reported partial interfertility between isolates of three collections of hydnoid (toothed) *P. pargamenus* from Quebec, New Brunswick and Pennsylvania. Although not considered by Macrae (1941), identical alleles may account for this partial fertility. With respect to *H. abietinus*, identical alleles have been reported by Macrae (1967) and Fries and Jonasson (1941).

A pattern of complete interfertility between isolates from

different collections of the same species is evidence for their conspecificity. For example, using the results of genetical tests collections, T360, T73, T592, T728 and T745 belong to the species, *H. pargamenus*, while T590, T589, T72, T122, T744 are identified as *H. subchartaceus*. These results support the delineations made using morphological features as criteria. However, the situation in which there is a non-reciprocal distribution of clamp connections has proved to be perplexing and the reasons for it are open to discussion. The term, "unilateral diploidization" was originally coined by Brodie (1948) and was later called "unilateral dikaryotization" by Raper (1966) because a binucleate or dikaryon cell was produced as a result of pairing and not a diploid cell containing a single fused nucleus. Diploidization is delayed until meiotic stages in the basidium of basidiomycetes. Brodie could advance no explanation for this phenomenon in *Cyathus stercoreus*. Work by Fulton (1950) on this species showed that unilateral dikaryotization was always associated with "blotchy" binucleate mycelium lacking clamp connections, and which acted as a nuclear acceptor. In *Schizophyllum commune* however, unilateral dikaryotization has been associated with common-A heterokaryons which acted as nuclear donors (Papazian, 1950, 1951; Raper, 1966). Morphologically aberrant isolates with gnarled mycelium, mutant isolates which mimic common factor heterokaryons in morphology and pairing reaction, and mycelia containing "modifier mutants" in which the expression of the mating alleles is affected have been reported in unilateral dikaryotizations of *S. commune* (Raper, 1966). All of these mycelia are uninucleate for the most part, and act as nuclear donors. Furthermore, Whitehouse (1949) points out that contaminant spores are always a problem in culture of monokaryotic

isolates. Considering the customary procedures used for the isolation of sporelings whereby spores are suspended in water, spread over the surface of agar media, and then picked off individually (Macrae, 1941, 1967), I believe that the possibility exists for the isolation of more than one spore. If A-loci were common to both, then largely uninucleate cells lacking clamps could indicate the existence of a slow-growing, monokaryotic mycelium.

Cases of unilateral dikaryotization in compatible pairings of *H. pargamenus* and *H. subchartaceus* have not been reported previously. They have, however, been reported by Fries and Aschan (1952) for *H. abietinus*. These workers observed that monokaryotic mycelia isolated by micro-surgery from a slow-growing, appressed, degenerate culture, 17 years old, produced clamp connections unilaterally and served as donors of nuclei in pairings with other monokaryons. The unilateral dikaryotizations that I have reported always involved morphologically aberrant isolates which were appressed and slow-growing compared to the other monokaryons. The compact, entangled and gnarled mycelium of these isolates served as donors of nuclei and remained mostly uninucleate themselves. Because this phenomenon was observed with increased frequency over a six-month period between intracollection pairings and intercollection pairings in some cases involving exactly the same isolates, it was concluded that the vigor and mating capacity of monokaryotic isolates was changing with age. The isolate of *H. abietinus* used by Fries and Aschan (1952) is a dramatic example of degeneration. Macrae (1941) used monokaryotic isolates of *H. pargamenus* that were less than a year to 9-years old, yet she did not report anomalies in the distribution of clamps. However, in that study she did not sample

mycelium on both sides of the interaction zone. Studies of other species have shown that monokaryons lose their vigor and mating capacity with time. For example, Lange (1952) claimed that isolates of *Coprinus* exhibited a general loss of vigor and a decline in the ability to mate with other monokaryons after prolonged storage. For *S. commune* "modifier mutants" are reported to occur with greater frequency in older mycelia and common-A heterokaryons (Raper, 1966). Moreover, the haploid nature of monokaryotic isolates enhances the expression of recessive mutants affecting the mating loci and undetected in dikaryon hyphae. Indirect support for the explanation that common-A heterokaryons are responsible for many of the unilateral dikaryotizations reported in this chapter is obtained from results of pairing known common-A heterokaryons of *H. pargamenus* (T360) with monokaryons. In these pairings, the appressed abnormal heterokaryon lacking clamped hyphae served as a nuclear donor to compatible isolate of T360 and other isolates of *H. pargamenus* collections. Although detailed studies of nuclear migration are needed, it appears that there is some barrier to nuclear migration into the hyphae of abnormal isolates. Furthermore, when two morphologically abnormal "monokaryotic" isolates are paired clamps and dikaryon cells are only formed where hyphae of the two isolates come into physical contact.

The intersterility of paired monokaryotic isolates of each species of *Hirschioporus* is somewhat ambiguous as a criterion for distinguishing *H. pargamenus* from *H. subchartaceus* because of the occurrence of "hybrid" mycelia. Rare hybrids were reported in pairings of *H. subchartaceus*, T590 and *H. pargamenus*, T592, T728, (four of 400 pairings). But, in 75 of the 80 pairings of *H. pargamenus*, T745 with *H. subchartaceus*

collections (Fig. 87) were fertile and displayed dikaryon cells with normal clamps at the junction or unilaterally. A similar result was reported for an "intermediate" collection of *P. pargamenus* (toothed) paired with poroid *P. pargamenus* by Macrae (1941). Other workers have reported such "hybrids" in *Peniophora* (Weresub and Gibson, 1960), *Pycnoporus* (Nobles and Frew, 1962), and *Coriolus* (Edwards and Kennedy, 1973), but they have generally been discounted in the overall pattern of intersterility of the species. In the case of *Peniophora* and *Coriolus* "hybrids", the hyphae became simple-septate with pseudoclamps and uninucleate cells after a few serial transfers (Weresub and Gibson, 1960; Edwards and Kennedy, 1973). Transfers of *Hirschioporus* "hybrids" indicate a similar degeneration to a simple-septate monokaryotic condition. As the above workers state, no authentic cases of hybridization have been reported for hymenomycetes and certainly, the successful fusion and dikaryon formation is no assurance of meiosis and spore formation in hybrids which have never been reported to fruit. "Hybrid inviability" is proposed by Burnett (1968) as an isolation mechanism in the speciation of fungi and appears to be operating here in the separation of these two species of *Hirschioporus*.

Demarcation lines between paired monokaryotic isolates is consistent evidence that two different *Hirschioporus* species are involved. These are white, submerged lines which are distinct from the mycelial interactions noted in intracollection pairings. Their occurrence was also reported in pairings of *Hirschioporus abietinus* and *H. fuscoviolaceus* (Robak, 1942). My view that they are identifying features in *Hirschioporus* does not agree with the opinions of Lange (1952) for *Coprinus* species and Edwards and Kennedy (1973) for *Coriolus* species.

As a further support for the opinion that demarcation lines have value in delimiting species of *Hirschioporus*, it must be emphasized that within this interaction zone dividing the colonies of different species conspicuous sickle-shaped clasping branches are very abundant. These modified hyphae are produced by all the *Hirschioporus* species examined in this thesis when confronted by each other or foreign species (Chapter VI). However, it is necessary to note the rare occurrence of clasping branches in intracollection pairings of abnormal and normal monokaryons of the same species. Similarly, they have been found in intercollection pairings of the same species involving the confrontation of dikaryon and monokaryon isolates from different collections, but were not observed in pairings of monokaryotic isolates from different collections of the same species. It seems that nuclear constitution and cytoplasmic compatibility are involved in the production of such structures when isolates from different collections of the same species are paired.

The production of clasping branches is not initiated at a distance by the action of diffusible substances but is found only on hyphae which have come in contact. However, further proof of this observation requires the separation of the mycelia by a semi-permeable membrane. Macrae (1967) has reported a type of antagonism between hyphae of *H. pargamenus* and *H. laricinus* in which short, swollen branches "embrace" contacted hyphae. Robak (1942) previously had reported this phenomenon for *H. laricinus* and *H. abietinus*. Griffith and Barnett (1967) observed a "necrotrophic mycoparasitism" for *P. pargamenus* in culture whereby host mycelia were killed and penetrated. However, the clasping branches reported here are more like the "absorptive structure" described for *Stephanoma phaeospora*, a contact mycoparasite which does not penetrate

the host (Rakvidhyasastra and Butler, 1973). In my study, killing of the mycelium and penetration was not observed with *Hirschioporus* species. Although parasitism is suggested, further evidence obtained from studies at the electron microscope level of possible penetration or alteration to the contacted host and of the morphology and development of the clasping branch, which resembles an appressorium, is required.

Pairings of dikaryon isolates of *H. abietinus*, *H. subchartaceus*, and *H. pargamenus* with monokaryotic isolates from different collections of these taxa (Fig. 89) have confirmed the results of interspecies sterility between *H. subchartaceus* and *H. pargamenus*, using the clamp criterion. In this case, T745 isolates are compatible only with other isolates from collections of *H. pargamenus*. However, the abnormal morphology of T435D12, a monokaryotic isolate of *H. abietinus* was associated with its failure to be dikaryotized by the dikaryon isolate of *H. abietinus*, T48. Generally speaking, I consider the Buller Phenomenon to be very useful as a genetical test for the identity of mycelia.

As indicated by the studies reported here for *Hirschioporus*, the genetical criteria used for the delimitation of species are not free from the ambiguities and exceptions found in any biological phenomenon. It has already been noted that the absence of clamp connections must be interpreted with caution as evidence for species identity. Contrary to what Lange (1952) has said, presence of clamps is also not always conclusive evidence that taxa are conspecific. In fact, the evaluation of the results of genetical tests illustrates some circular thinking. For example, the boundaries set by genetical criteria are evaluated in terms of their conformity to the delimitations made using morphological

criteria. It is hardly necessary to emphasize the need for information to support the results of genetical studies based on the single character, presence or absence of clamp connections. Moreover, classification of polypores must be based on a sound understanding of the interrelationship of species and genera. Such an understanding requires comparisons using many characteristics from all stages of the life-cycle; this requirement is not satisfied by genetical studies alone.

TABLE 4. Collections used in genetical studies

Species	Collection*	Substrate	Geographic Location
H. pargamenus	T728	*Betula*	What Cheer, Iowa
	T592	*Betula*	Edmonton, Alberta.
	T360	*Betula*	Winterburn, Alberta
	T73	*Betula*	New Norway, Alberta
	T745	*Quercus*	Ann Arbor, Michigan
H. subchartaceus	T72	*Populus*	New Norway, Alberta
	T590	*Populus*	Wabamun Lake, Alberta
	T122	*Populus*	Borden, Saskatchewan
	T589	*Populus*	Grande Prairie, Alberta
	T744	*Prunus*	Ann Arbor, Michigan
H. abietinus	T435	*Picea*	Prophet River, British Columbia
	T587	*Picea*	Grande Prairie, Alberta
	T586	*Abies*	Carson Lake, Alberta
	T48	*Picea*	Little Smoky, Alberta

*Fruiting strains.

In Figures 74-89, the following symbols are used:

+ represents bilateral distribution of normal clamps

$+_u$ represents unilateral distribution of normal clamps

p represents the formation of pseudoclamps at the junction of paired isolates

- represents the absence of clamp connections on hyphae

* represents the formation of normal clamps only at the junction of paired mycelia.

FIGURE 74. Monospore isolates of *H. pargamenus*, T728 paired in all combinations. The four mating types are designated as A_1B_1, A_2B_2, A_1B_2 and A_2B_1.

FIGURE 75. Monospore isolates of *H. pargamenus*, T592 paired in all combinations. Note the rare occurrence of unilateral dikaryotization.

T728

T728	1	9	5	11	7	14	6	4	8	10	3	
1		−	−	−	+	+	+	+	p	p	p	A_1B_1
9	−		−	−	+	+	+	+	p	p	p	
5	−	−			p	p	p	p	+	+	+	A_1B_2
11	−	−	−		p	p	p	p	+	+	+	
7	+	+	p	p		−	−	−	−	−	−	
14	+	+	p	p	+		−	−	−	−	−	A_2B_2
6	+	+	p	p	−	−		−	−	−	−	
4	+	+	p	p	−	−	−		−	−	−	
8	p	p	+	+	−	−	−	−		−	−	
10	p	p	+	+	−	−	−	−	−		−	A_2B_1
3	p	p	+	+	−	−	−	−	−	−		

74

T592

T592	14	16	17	19	25	28	2	29	26	8	5	21	6	27	
14		−	−	−	−	+	+	+	+	+	p	−	−	p	
16	−		−	−	−	+	+	+	+	+	−	p	p	p	A_1B_1
17	−	−		−	−	+	+	$+_u$	+	+	p	p	p	p	
19	−	−	−		−	p	−	p	p	p	+	+	+	+	
25	−	−	−	−		−	−	p	p	p	+	+	+	+	A_1B_2
28	+	+	+	p	−			−	−	−	−	−	−	−	
2	+	+	+	−	−	−		−	−	−	−	−	−	−	
29	+	+	$+_u$	p	p	−	−		−	−	−	−	−	−	A_2B_2
26	+	+	+	p	p	−	−	−		−	−	−	−	−	
8	+	+	+	p	p	−	−	−	−		−	−	−	−	
5	p	−	p	+	+	−	−	−	−	−		−	−	−	
21	−	p	p	+	+	−	−	−	−	−	−		−	−	
6	−	p	p	+	+	−	−	−	−	−	−	−		−	A_2B_1
27	p	p	p	+	+	−	−	−	−	−	−	−	−		

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FIGURE 76. Monospore isolates of *H. subchartaceus*, T72 paired in all combinations.

FIGURE 77. Monospore isolates of *H. subchartaceus*, T590 paired in all combinations.

T72

T72	7	14	17	2	11	6	4	8	3	18	5	15	
7		+	$+_u$	+	+	+	−	−	−	p	p	p	A_1B_1
14	+		−	−	−	−	−	p	p	−	−	−	
17	$+_u$	−		−	−	−	−	p	p	−	−	−	
2	+	−	−		−	−	p	p	p	−	−	−	A_2B_2
11	+	−	−	−		−	p	p	p	−	−	−	
6	+	−	−	−	−		−	p	−	−	−	−	
4	−	−	−	p	p	−		−	−	*	+	*	
8	−	p	p	p	p	p	−		−	$+_u$	+	$+_u$	A_1B_2
3	−	p	p	p	p	−	−	−		*	+	+	
18	p	−	−	−	−	−	*	$+_u$	*		−	−	
5	p	−	−	−	−	−	+	+	+	−		−	A_2B_1
15	p	−	−	−	−	−	*	$+_u$	+	−	−		

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T590

T590	15	20	5	14	8	16	17	1	4	3	19	9	11	25	18	
15		−	−	−	+	+	+	$+_u$	+	−	−	−	−	−	−	
20	−		−	−	+	+	+	$+_u$	+	−	−	−	−	−	−	A_1B_1
5	−	−		−	+	+	+	$+_u$	+	−	−	−	−	−	−	
14	−	−	−		+	+	+	$+_u$	+	−	−	−	−	−	−	
8	+	+	+	+		−	−	−	−	p	p	p	p	p	p	
16	+	+	+	+	−		−	−	−	p	p	p	−	p	p	
17	+	+	+	+	−	−		−	−	p	p	p	p	−	p	A_2B_2
1	$+_u$	$+_u$	$+_u$	$+_u$	−	−	−		−	p	p	p	p	p	−	
4	+	+	$+_u$	+	−	−	−	−		p	p	p	p	p	p	
3	−	−	−	−	p	p	p	p	p		−	−	−	−	−	
19	−	−	−	−	p	p	p	p	p	−		−	−	−	−	
9	−	−	−	−	p	p	p	p	p	−	−		−	−	−	A_1B_2
11	−	−	−	−	p	−	p	p	p	−	−	−		−	−	
25	−	−	−	−	p	p	−	p	p	−	−	−	−		−	
18	−	−	−	−	p	p	p	−	p	−	−	−	−	−		

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FIGURE 78. Pairings which show compatible pairs of monokaryons (+) in collections of *H. pargamenus*, T360, T73, T745 and *H. subchartaceus*, T744, T589, T122.

T360

	18	10	
4	−	+	
9	−	+	
19	−	+	
20	−	+	A_1B_2
13	−	+	
23	−	+	
17	−	+	
8	+	−	
6	+	−	A_2B_2
1	+	−	
21	−	p	
18		p	A_1B_1
15	p	−	
10	p	−	A_2B_1
24	p	−	
14	−	−	
3	−	−	
11	−	−	
22	−	−	
5	−	−	
7	−	−	

T122

	8	14
14	−	
8		−
11	−	−
12	−	−
9	$+_u$	p
7	+	p
6	−	+

T73

	4	13
14	−	−
13	−	
10	−	−
3	−	−
1	−	−
12	−	−
16	−	−
20	−	−
4		−
17	−	−
11	−	+
2	−	+
19	−	+
6	−	+
15	−	+
8	−	+
9	+	p

T745

	1	9
12	−	−
8	−	−
13	−	−
9	−	
10	+	+
7	+	+
3	p	p
6	p	p
11	p	p
5	p	p

T589

	16	10
2	−	−
23	−	−
22	−	−
12	−	−
1	−	−
16		−
10	−	
21	+	+
17	−	p
3	−	p
5	−	p

T744

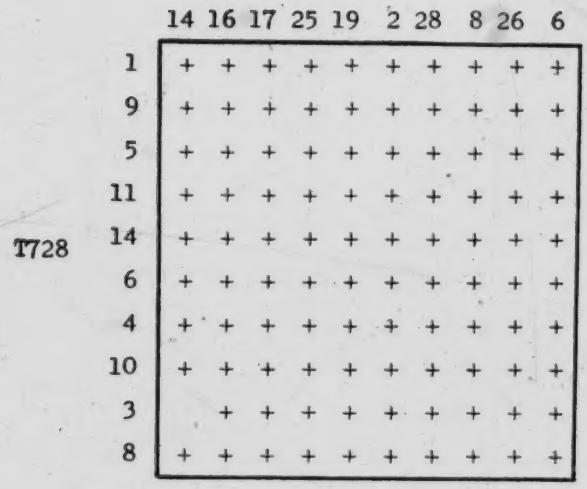
	10	21
1	+	+
7	+	+
17	+	+
20	+	+
5	+	+
6	−	−
8	−	−
16	−	−
14	−	−
11	−	−
4	−	
15	−	
21	−	
10		−
13	p	p
2		p
12		+

FIGURE 79. Intraspecies pairings of monospore isolates from two collections of *H. pargamenus*, T592 and T728.

FIGURE 80. Intraspecies pairings of monospore isolates from two collections of *H. subchartaceus*, T590 and T72.

T592

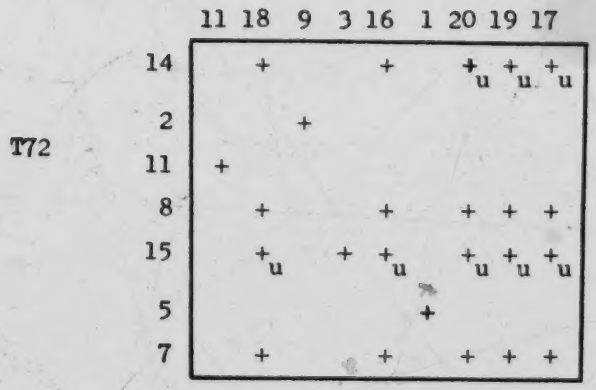
T728



79

T590

T72



80

FIGURE 81. Interspecies pairings of monospore isolates from collections of *H. pargamenus*, T592 and *H. subchartaceus*, T72.

FIGURE 82. Interspecies pairings of monospore isolates from collections of *H. pargamenus*, T592 and *H. subchartaceus*, T590. Note the rare occurrence of "hybridization".

T592

T72	14	16	17	25	19	2	28	8	26	6
7	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-

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T592

T590	6	26	8	28	2	19	25	17	16	14
14	-	-	-	-	-	-	-	-	-	-
20		-		-		-		-	-	-
18	-	-	-	-	-	-	-		+u	-
9	-	-		-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-		-	-	-	-	
16		-	-	-	-	-	-	-	-	-
17			-		-	-	-	-		-
1	-	-	-	-	-	-	-	-	-	-

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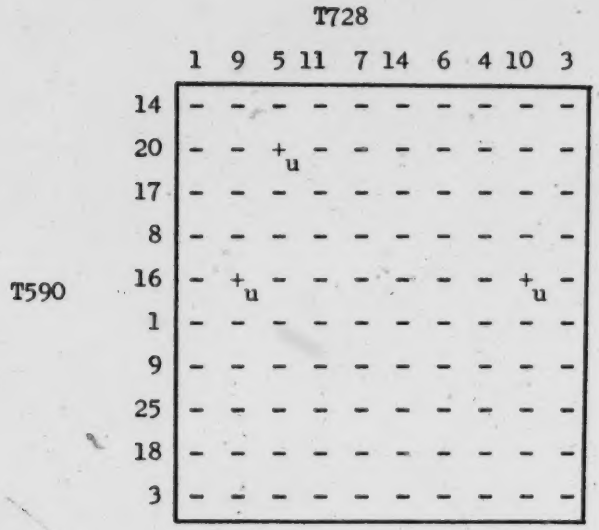
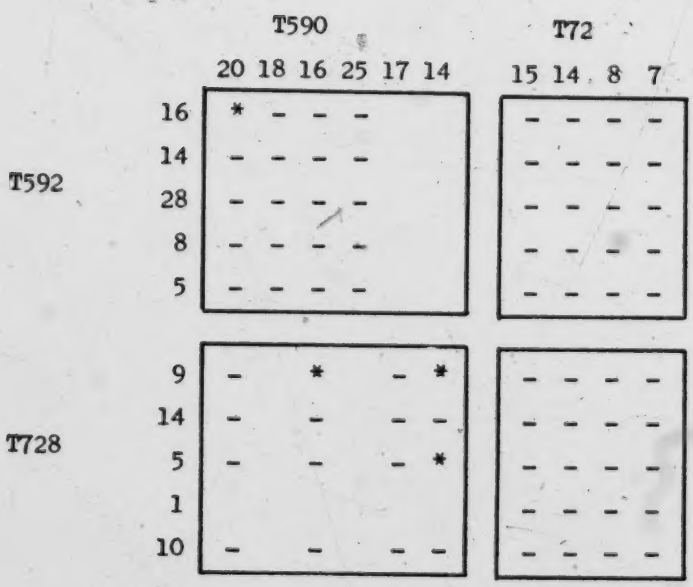
FIGURE 83. Interspecies pairings of monospore isolates from collections of *H. pargamenus*, T728 and *H. subchartaceus*, T590.

FIGURE 84. Interspecies pairings of monospore isolates from two collections of *H. subchartaceus*, T590 and T72 and two collections of *H. pargamenus*, T592 and T728.


T728
1 9 5 11 7 14 6 4 10 3
T590
14
20
17
8
16
1
9
25
18
3

83


T590
20 18 16 25 17 14
T72
15 14 8 7
T592
16
14
28
8
5
T728
9
14
5
1
10

84

FIGURE 85. Interspecies pairings of monospore isolates from five collections of *H. pargamenus*, T360, T73, T592, T728, T745. Within each collection two compatible pairs of isolates were selected for pairing.

		T360				T73				T592				T728				T745			
		18	21	6	8	13	9	11	4	16	14	28	8	9	1	14	7	1	9	10	7
T360	18					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	21					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	8					$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$
T73	13	+	+	+	$+_u$					$+_u$	+	+	+	+	+	+	+	+	+	+	+
	9	+	+	+	$+_u$					$+_u$	$+_u$	+	+	$+_u$	+	+	+	+	+	*	+
	11	+	+	+	$+_u$					$+_u$	$+_u$	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	$+_u$					$+_u$	$+_u$	+	+	*	$+_u$	$+_u$	$+_u$	+	+	+	*
T592	16	+	+	+	$+_u$	$+_u$	$+_u$	$+_u$	$+_u$					+	+	+		+	+	+	+
	14	+	+	+	$+_u$	+	$+_u$	$+_u$	$+_u$					+	+	+		+	+	+	+
	28	+	+	+	$+_u$	+	+	+	+					+	+	+		+	+	+	+
	8	+	+	+	$+_u$	+	+	+	+					+	+	+		+	+	+	+
T728	9	+	+	+	$+_u$	+	$+_u$	+	*	+	+	+	+					+	+	+	*
	1	+	+	+	$+_u$	+	+	+	$+_u$	+	+	+	+					+	+	+	+
	14	+	+	+	$+_u$	+	+	+	$+_u$	+	+	+	+					+	+	+	+
	7	+	+	+	$+_u$	+	+	+	$+_u$									+	+	+	+
T745	1	+	+	+	$+_u$	+	+	+	*	+	+	+	+	+	+	+	+				
	9	+	+	+	$+_u$	+	+	+	+	+	+	+	+	+	+	+	+				
	10	+	+	+	$+_u$	+	*	+	+	+	+	+	+	+	+	+	+				
	7	+	+	+	$+_u$	+	+	+	*	+	+	+	+	*	+	+	+				

FIGURE 86. Intraspecies pairings of monospore isolates from five collections of *H. subchartaceus*, T590, T589, T72, T122, T744.

		T590				T589				T72				T122				T744			
		16	17	20	15	16	10	21	17	8	18	14	7	8	14	7	6	10	21	1	7
T590	16					+	+	+	+u	+		+	+	+	+	+u	+	+	+u	+	+
	17					+	+u	+u	+u	+		+u	+	+	+	+u	+	*	+u	*	+
	20					+	+	+	+u	+		+u	+	+	+	+u	+	+	+u	+	+u
	15					*	*	*	*					+u	+u	*	+u	*	+u	+u	+u
T589	16	+	+	+	*					+u	+u	+u	+u	+	+	+u	+	+u	+u	+	+u
	10	+	+u	+	*					+	+u	+u	+	+u	+	+u	+	+	+	+u	+u
	21	+	+u	+	*					+	+u	+u	+	+	+	+	+	+u	+u	+u	+u
	17	+u	+u	+u	*					*	+u	+u	+	+	+	+	+	+u	+u	+u	+u
T72	8	+	+	+		+u	+	+	*					+	+	+u	+	+	+	+	+
	18					+u	+u	+u	+u					+	+	+u	+	+u	+u	+u	+u
	14	+	+u	+u		+u	+u	+u	+u					+	+	+u	+	+u	+u	+u	+u
	7	+	+	+		+u	+	+	+					+	+	+	+	+	+	+	+
T122	8	+	+	+	+u	+	+u	+	+	+	+	+	+					+	+	+	+
	14	+	+	+	+u	+	+	+	+	+	+	+	+					+	+	+	+
	7	+u	+u	+u	*	+u	+u	+	+	+u	+u	+u	+					+u	+u	+u	+u
	6	+	+	+	+u	+	+	+	+	+	+	+	+					+	+	+	+
T744	10	+	*	+	*	+u	+	+u	+u	+	+u	+u	+	+	+	+u	+				
	21	+u	+u	+	+u	+u	+	+u	+u	+	+u	+u	+	+	+	+u	+				
	1	+	*	+	+u	+	+u	+u	+u	+	+u	+u	+	+	+	+u	+				
	7	+	+u	+u	+u	+u	+u	+u	+u	+	+u	+u	+	+	+	+u	+				

FIGURE 87. Interspecies pairings of monospore isolates from five collections of *H. pargamenus* (T360, T73, T592, T728, T745) and five collections of *H. subchartaceus* (T590, T489, T72, T122, T744). Note the frequent occurrence of hybridization involving isolates of *H. pargamenus*, T745.

		T360				T73				T592				T728				T745			
		18	21	6	8	13	9	11	4	16	14	28	8	9	1	14	7	1	9	10	7
T590	16	–		–	–	–	–	–	–	–	–	–	–	*		–		*	*	*	*
	17	–	–	–	–	–	–	–	–					–		–		*	*	*	*
	20	–	–	–	–	–	–	–	–	*	–	–	–	–		–		*	*	*	*
	15	–	–	–	–	–	–	–	–									*	–	*	*
T589	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	$+_u$	–
	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*	*
	21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*	–
	17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	$+_u$	–
T72	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		*	*	*	*
	18	–	–	–	–	–	–	–	–									*	*	*	*
	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		*	*	*	*
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		*	$+_u$	*	*
T122	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*	*
	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*	–
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	*	*
T744	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	$+_u$	$+_u$	$+_u$	*
	21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	$+_u$	*	*	*
	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	$+_u$	*	$+_u$	*
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	$+_u$	*	*

FIGURE 88. Pairings of a common-A heterokaryon of *H. pargamenus* (T360-18 + T360-4) with monokaryons of T360 and other collections of *H. pargamenus*, T728 and T592 and of *H. subchartaceus*, T590, T589, T122, T72.

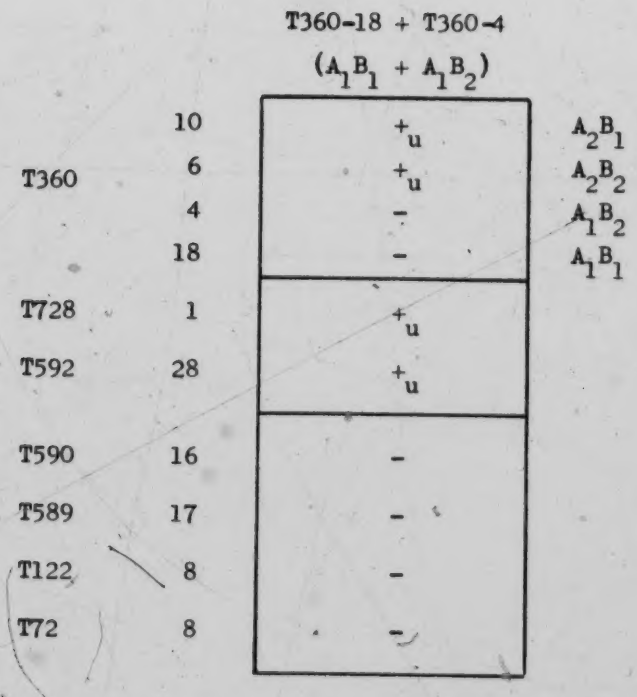
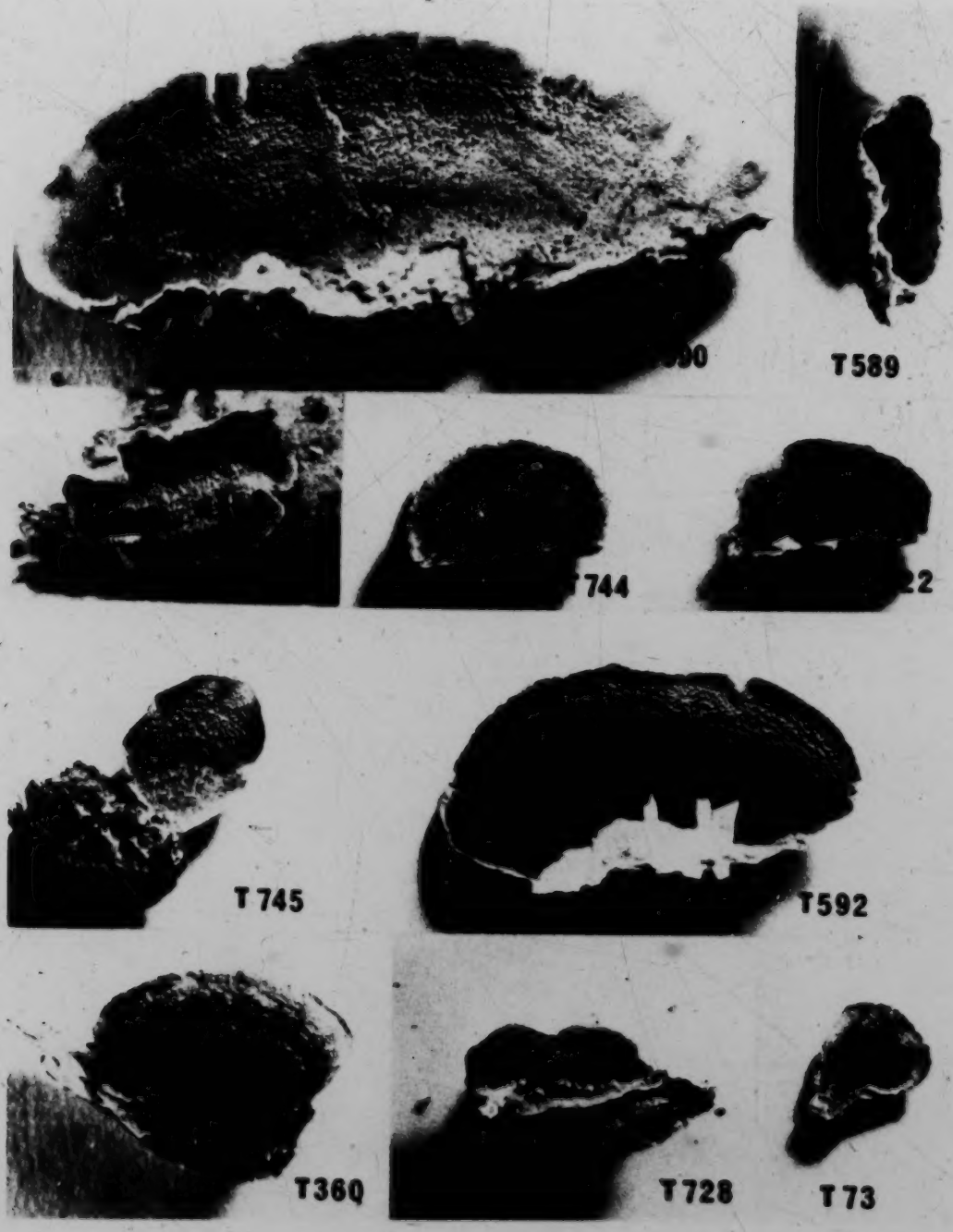

T360-18 + T360-4
(A1B1 + A1B2)
10
6
4
18
1
28
16
17
8
8
T360
T728
T592
T590
T589
T122
T72
+u
+u
-
-
+u
+u
-
-
-
-
A2B1
A2B2
A1B2
A1B1

FIGURE 89. The Buller Phenomenon.

Pairings of dikaryon isolates from collections of *H. pargamenus* (T360, T745), *H. subchartaceus* (T122) and *H. abietinus* (T48) with monospore isolates from collections of *H. subchartaceus* (T593, T72, T122, T590, T589, T744), *H. pargamenus* (T745, T73, T728, T360, T592), and *H. abietinus* (T587, T435, T586).

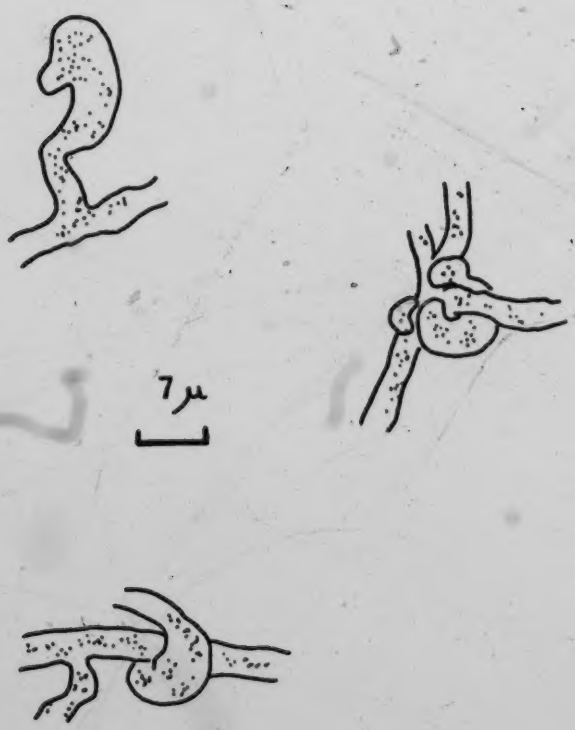
		T360	T745	T122	T48
T593	13	–	–	+	–
T72	7	–	–	+	–
T122	14	–	–	+	–
T590	16	–	–	+	–
T589	10	–	–	+	–
T744	7	–	–	+	–
	6	–	–	+	–
T745	9	+	+	–	–
T73	11	+	+	–	–
	13	+	+	–	–
T728	7	+	+	–	–
T360	6	+	+	–	–
	18	+	+	–	–
T592	18	+	+	–	–
	8	+	+	–	–
T587	4	–	–	–	+
T435	12	–	–	–	+
T586	17	–	–	–	+

FIGURE 90. Hymenial surfaces of basidiocarps from collections of *H. subchartaceus* (top five) and *H. pargamenus* (bottom five) used in genetical studies. X1.4



90

FIGURE 91. Clasping branches. Swollen, sickle-shaped structures on the hyphae of *Hirschioporus* species in the interaction zone between mycelia of different taxa.



91

FIGURE 92. Appressed, slow-growing common-A heterokaryon of *H. pargamenus* (T360-18 + T360-4). The mycelium acts as nuclear donor to isolate, T360-10 which surrounds it. X0.75

FIGURE 93. Intraspecies pairing of monospore isolates from collections of *H. subchartaceus*, T72 (top) and T590 (bottom) after 3 weeks. Note the absence of any demarcation line. X0.75

FIGURE 94. Intraspecies pairing of monospore isolates from collections of *H. pargamenus*, T728 (top) and T592 (bottom) after 3 weeks. X0.75

FIGURE 95. Interspecies pairing of monospore isolates from a collection of *H. subchartaceus*, T72 (top) and *H. pargamenus*, T592 (bottom). Note the white line of demarcation. X0.75.

FIGURE 96. Comparison of a normal dikaryon of *H. pargamenus* with "hybrid" after two weeks growth on Nobles' malt agar. The felt-like to tufted colony of *H. pargamenus*, T73 + T745 (top plate) shows a faster growth rate than the appressed, plumose colony of the "hybrid", T745 + T590 (bottom). X0.6

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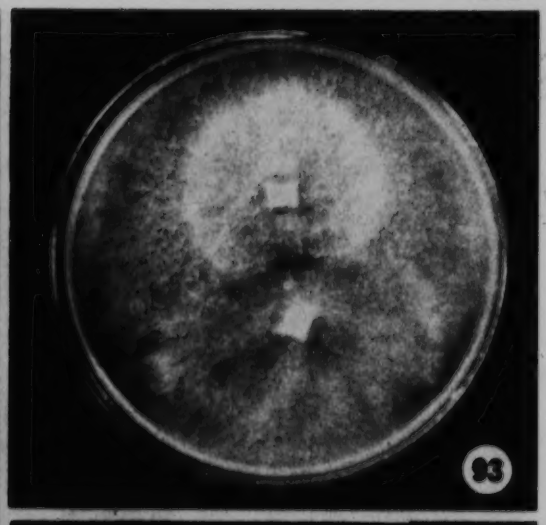
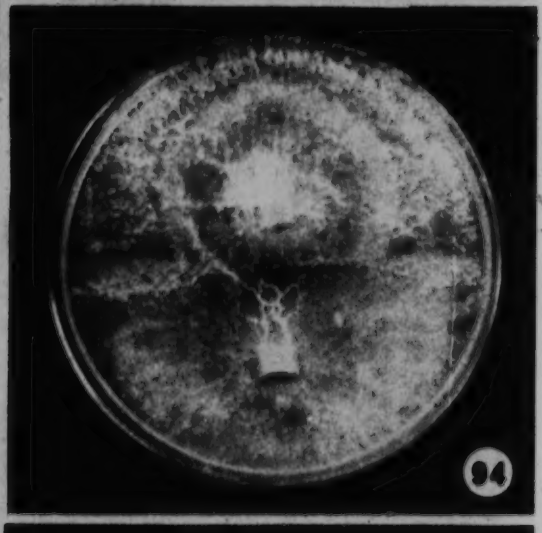
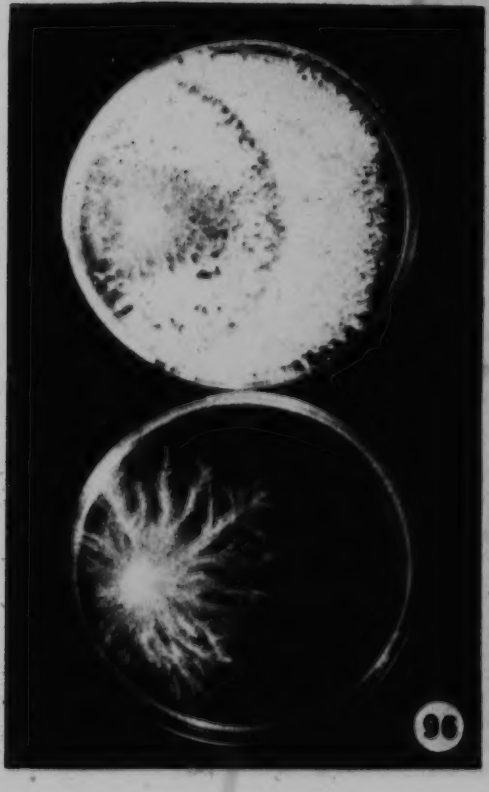

93


94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CHAPTER VI

MYCELIAL INTERACTIONS IN MIXED CULTURE

Introduction

An interest in the mycelial interactions of *Hirschioporus* species was stimulated by the observation of white demarcation lines and clasping branches in interspecies confrontations of monokaryotic isolates. Such responses provided important information in support of the identification of these species by the clamp criterion.

Various workers have reported coloured zones between dikaryotic isolates of different species of polypores, but these studies have not involved *Hirschioporus* species. For example, Suverov (1970) described "sharp boundaries" between dikaryon colonies of five *Fomes* species paired on malt agar. Henningsson (1967) reported blue zones between colonies of *Coriolus* (*Polyporus*) *versicolor*, *C. hirsutus*, and *C. zonatus* paired with *Stereum purpureum* (Thelephoraceae), but yellow-brown zones between these fungi and *Stereum hirsutum*. It was noted that coloured zones in mixed cultures were very similar to "coloured zones" in aspen and birch wood decayed by *S. purpureum* (Henningsson, 1967).

At present, more than one explanation exists for the general phenomenon, production of coloured zones of "zone lines" in decayed wood. Because there is no conclusive evidence that justifies the equivalence of reactions between mycelia in nature (in decaying wood) with mycelial interactions in culture, the term "zone lines" has been reserved for the dark, blackish lines observed in wood and coloured zones is used for

phenomena on malt agar. Rhoads (1918) stated that zone lines produced in wood decayed by *P. pargamenus* were the result of oxidation products in dead wood cells and thus were found in advance of the growth of the decay organism. Hubert (1924) listed six wood-decay fungi which produced "zone lines" in agar and wood as a response of the mycelium to evaporative conditions. These species included *Trametes pini*, *Fomes igniarius*, *Xylaria polymorpha* (Ascomycete), *Polyporus adustus*, *Hymenochaete ruginosa* and *Ganoderma curtsii*. Hopp (1938) reported black "zone lines" in pure cultures on malt agar and wood, of *Fomes applanatus*, *F. fomentarius*, *F. fraxinophilus*, *F. igniarius* and *Polyporus hispidus* in response to aeration and drying conditions. His experiments showed that the hyphae in the "zone line" were gnarled or swollen, pigmented and compactly tangled, forming a barrier between very moist and drier conditions. Similarly, Campbell and Munson (1936) described the "zone lines" of *Polyporus squamosus* in malt agar and wood cultures as a "pseudosclerotium" because of the rind-like properties of the pigmented, "bladder hyphae". Another explanation for zone lines is that they are formed "at the point of contact of two invading fungi" (White, 1920). Hubert (1924), for example, reported zone lines between *Polyporus anceps* and *Lenzites* (*Gloeophyllum*) *saepiaria*, and between *Fomes applanatus* and *Stereum frustulatum*. Although Hubert cited zone lines as diagnostic features of the decay produced by *Polystictus* (*Hirschioporus*) *pargamenus* and *P. abietinus*, he did not indicate their nature or cause.

Antagonism between wood-decay fungi has been studied by a few mycologists. Such studies have shown that ascomycetes inhibit the growth of basidiomycetes in malt agar culture and that their prior colonization of wood in culture reduces subsequent colonization and decay

by basidiomycetes. For example, *Fomes pini* was inhibited by the ascomycetes, *Tympanis hypopodia* (Basham, 1966) and *Ascocoryne sarcoides* (Basham, 1973; Whittaker, 1962). *Coniophora puteana* (Thelephoraceae) and *Polyporus tomentosus* decay were inhibited by *Coryne (Ascocoryne) sarcoides* (Etheridge, 1957) with which the wooden blocks had been inoculated previously. Cultural studies by Basham (1966, 1973) showed also that coloured zones were produced at the junction of the colonies of the different species. These reports deal with chemical inhibitions. Another kind of antagonism involving basidiomycetes in culture has been reported by Griffith and Barnett (1967), Robak (1942) and Macrae (1967). Necrotrophic mycoparasitism was described for *P. pargamenus* (Griffith and Barnett, 1967), while Robak (1942) and Macrae (1967) described swollen branches of *H. laricinus* and *H. pargamenus* entwining hyphae of other *Hirschioporus* species. As discussed in the previous chapter, these structures are probably the clasping branches which I have described in the genetical studies.

Different polypore species were observed fruiting with *Hirschioporus* species on the same logs in close proximity to each other. Assuming that there could be mycelial interaction, isolates from selected polypore associates were paired on malt agar with dikaryon isolates of the three *Hirschioporus* species. It was observed that different polypore species were associated with each *Hirschioporus* species in the field. *Trametes hispida* and *Cerrena unicolor* were selected as commonly occurring with *H. subchartaceus* and *H. pargamenus*, respectively. *Gloeophyllum saepiarium* and *Fomes pini* were selected as commonly occurring in the wood decayed by *H. abietinus*. These four species were paired with the three species of *Hirschioporus* in order to ascertain the differences in response and to

assess the taxonomic significance of any differences.

Materials and Methods

Dikaryotic isolates obtained from wood beneath the basidiocarps of *Hirschioporus* species, *Trametes hispida*, (T520), *Cerrena unicolor*, (T523), *Fomes pini*, (T540) and *Gloeophyllum saepiarium*, (T15) were opposed on Nobles' malt agar in plastic petri plates. Pairings were made between dikaryon isolates of the four selected species from different genera and three dikaryon isolates for each of *H. pargamenus* (T73, T592, T30), *H. subchartaceus* (T526, T588, T590) and *H. abietinus* (T532, T58, T48). For each pairing, four plates containing 20 ml of medium were inoculated six cm apart with 5 mm discs of agar and mycelium taken from the margin of 10-day-old cultures on NMA. Three plates were wrapped separately in aluminum foil, placed in plastic bags and incubated in a controlled temperature chamber at a constant 25°C. Sample plates were removed at three weekly intervals. A fourth series of pairings was stored in plastic bags on the lab bench at 22°C in alternating light and dark conditions. For microscopic observations, small pieces of agar and mycelium were squash-mounted in distilled water or KOH-phloxine. Also mycelium in blocks of agar (1 mm square) was examined directly under the microscope.

Paired colonies of the same isolates for each species were examined as controls. Moreover, dikaryon isolates of different *Hirschioporus* species were paired in all combinations as were dikaryon isolates of the four selected species from other genera.

Observations

Paired mycelia interacted at the junction of the colonies and produced a response which was characteristic of a particular combination. This region of mycelial contact is termed the interaction zone. One component of the pairing was often more aggressive and grew over the other. In this case, the interaction zone moved in advance of the aggressive isolate. Results for experiments involving different isolates of *Hirschioporus* species paired with isolates of different genera were similar in plates incubated in darkness and plates stored in alternating light and dark conditions. Results of these pairings, pairings between different *Hirschioporus* species and pairings between the four selected associates are summarized in Table 5 and described in more detail here.

Interactions of *Hirschioporus* species

At the junction of colonies of different *Hirschioporus* species white, submerged lines of demarcation were observed. These lines, most obvious between *H. pargamenus* and *H. subchartaceus* (Fig. 100), consisted of aerial matted and submerged, much branched, interwoven hyphae as seen in paired mycelia in genetical tests (Chapter V). No pigmentation was observed in the interaction zone and species did not grow over each other. Within the junction branches from thin-walled hyphae of each species wrapped around hyphae of the opposing colonies. These swollen, (4-6 μ wide), sickle-shaped branches whose external diameters were 10-12 μ, were attached to thin-walled hyphae (2-3 μ in diameter). They did not penetrate or alter the contacted mycelium and were densely cytoplasmic. Empty clasping branches were observed, but the contacted hyphae were also dead and free of cell contents.

Interactions of species other than *Hirschioporus*

(a) *Cerrena unicolor* and *Trametes hispida*

In the above combination colonies did not grow over each other. At the junction a yellowish diffusion zone was produced containing irregularly contorted and gnarled hyphae of *Trametes hispida* incrusted by fine yellow granules. Granular material staining with phloxine was observed as extruded globules from the tips of *C. unicolor* hyphae. A distinct, dark blue zone was observed between the colonies. The blue colour was in the agar and dispersed with squash-mounting in water. Crystal-incrusted hyphae were not observed in the blue region.

(b) *Cerrena unicolor* and *Gloeophyllum saepiarium*

G. saepiarium was overgrown in a wide band (2-3 cm), appearing felted and white on the surface and yellowish in the reverse. *G. saepiarium* hyphae reacted to this overgrowth by becoming contorted and gnarled. The yellow colouration was due to yellowish, granular material in the cytoplasm of the *G. saepiarium* hyphae. *Cerrena unicolor* mycelium in the interaction zone was gnarled, and terminal extrusions of cytoplasmic substances from thin-walled hyphae were observed.

(c) *Cerrena unicolor* and *Fomes pini*

Fomes pini colonies were completely over-grown by the mycelium of *C. unicolor*. The aerial mycelium in this overlapping region was cottony to felted and denser than in pure cultures of *C. unicolor*. A yellowish pigmentation was noted in the reverse of *F. pini* colonies near the interaction zone. In this area the hyphae of *F. pini* which lacked clamps, were much branched and contorted and contained yellowish granular cytoplasm.

(d) *Trametes hispida* and *Gloeophyllum saepiarium*

Trametes hispida grew over *G. saepiarium* producing a band (2-3 cm

wide) of felted, white mycelium containing abundant thick-walled hyphae. Within the agar of this region and that of the interaction zone the hyphae of both species were gnarled and much entangled. Yellowish pigmentation seen in the reverse was due to the yellow, granular cytoplasm of *G. saepiarium* hyphae (which were hyaline in pure culture).

(e) *Trametes hispida* and *Fomes pini*

Mycelium of *T. hispida* partly overgrew the *F. pini* colonies and produced a felted, white surface mat. *Fomes pini* reacted by producing intensely pigmented, yellow-brown hyphae in a narrow dark brown zone separating the colonies. The yellowish material diffused into the agar. The hyphae of *Trametes hispida* became conspicuously gnarled and vesiculate in this yellow-brown interaction zone.

(f) *Gloeophyllum saepiarium* and *Fomes pini*

Mycelium of *G. saepiarium* was less successful in growing over *F. pini*. In the interaction zone a dark brown area was produced at the margin of *F. pini* colonies. Hyphae of *F. pini* contained granular, yellowish-brown contents and formed distinct knots cemented together by incrustations of this yellowish material. *G. saepiarium* hyphae also became yellowish and, in the interaction zone, produced blobs of extruded cytoplasm at their apices.

Interactions of *Hirschioporus* species with species of other genera

(a) Pairings with *Trametes hispida*

Colonies of *Trametes hispida* grew over all three *Hirschioporus* species and produced a white, felt-like surface mat. In confrontations between *T. hispida* and *H. pargamenus*, in particular, the interaction zone was yellow and contained a narrower, blue zone (Figs. 97, 98, 105). The yellow colouration was produced by the presence of yellowish, crystal-

incrusted hyphae of *T. hispida*, whereas the blue zone was caused by a pigment diffused in the agar and concentrated in a narrow band which separated the two colonies. Clasping branches on the hyphae of *Hirschioporus* were observed in the interaction zone where hyphae of both species were tightly entwined. The extent of overgrowth was greater in pairings with *H. subchartaceus* and *H. abietinus* mycelia than in pairings with isolates of *H. pargamenus*. The yellow zone and blue band were not observed in the interaction zone of confrontations with *H. abietinus* (Fig. 105). In pairings with *H. subchartaceus* and *H. abietinus*, clasping branches were infrequently observed. Blobs of extruded, cytoplasmic material were observed at the tips and along the length of the hyphae in the mycelium of *Hirschioporus* spp. underlying *T. hispida* mats. The presence of cystidia in this mycelium identified it as belonging to the *Hirschioporus* isolate.

(b) Pairings with *Cerrena unicolor*

As with *Trametes hispida*, the colonies of *Hirschioporus* species were overgrown by the rapidly advancing mycelia of *Cerrena unicolor*. *H. pargamenus* was, however, more resistant to this overgrowth than the other two species (Fig. 107). A narrow, submerged line of demarcation was noted in a pale yellowish interaction zone (2-3 mm wide). Sickle-shaped branches of *H. pargamenus* clasped hyphae of *C. unicolor* in this zone. The extent of overgrowth of *H. subchartaceus* and *H. abietinus* (1-2 cm) by *C. unicolor* was marked by denser, white aerial growth and no colour change in the agar. Demarcation lines containing submerged, entwined hyphae, but few clasping branches, were observed. Gnarled, contorted hyphae as well as globular, cytoplasmic extrusions (Figs. 103, 104) were frequently noted in the interaction zone.

(c) Pairings with *Fomes pini*

All three species of *Hirschioporus* reacted similarly to mycelia of *Fomes pini*. Abundant clasping branches were observed (Figs. 101, 102) wrapped around the granular yellowish hyphae of *Fomes pini* within a yellowish-brown interaction zone. The demarcation zones were very dark, yellowish brown (Figs. 99, 106) due to the many hyphae of *F. pini* entangled in this region. A second dark zone near the inoculum where the agar surface had been cut, contained gnarled, inflated yellow-brown hyphae with granular contents.

(d) Pairings with *Gloeophyllum saepiarium*

H. pargamenus and *H. subchartaceus* colonies were overgrown by *G. saepiarium* in a narrow band (10-15 mm) of felted, tan mycelium appearing yellowish in reverse. *Hirschioporus* hyphae under this zone were empty and collapsed. The hyphae of *Gloeophyllum saepiarium* with their yellowish contents were entwined by hyaline *Hirschioporus* hyphae. Clasping branches were frequently observed in confrontations with *H. pargamenus*. However, coloured demarcation zones were most obvious in pairings with *H. abietinus*. In the interaction zone the dark brown zone consisted of collapsed, contorted thin-walled hyphae entangled by yellowish granular hyphae of *G. saepiarium*.

Discussion

Coloured zones in mixed cultures are useful in distinguishing these three *Hirschioporus* species. For example, confrontation of *H. pargamenus* and *H. subchartaceus* by mycelia of *Trametes hispida* results in a yellow interaction zone containing a dark blue band of demarcation. This reaction is most intense in pairings that involve *H. pargamenus*.

Confrontations with mycelia of *Cerrena unicolor* result in yellow interaction zones with isolates of *H. pargamenus* only. In confrontations with *G. saepiarium* the interaction zone between it and *H. abietinus* is characterized by a dark brown zone, whereas that of *H. pargamenus* and *H. subchartaceus* is faintly yellowish.

These responses however are largely due to the reactions of the species in the genera other than *Hirschioporus*. For example, pairing of *Trametes hispida* and *Cerrena unicolor* also resulted in the production of a yellow interaction zone containing a narrower blue band between the colonies. The conclusion that such zones are useful in the identification of species is not in agreement with the findings of Adams and Roth (1967) for *Fomes cajanderi* where coloured zones were found in paired cultures of different strains (dikaryotic) of the same fungus. Similarly, Edwards (1970) reported pigmented zones in intraspecies pairings of monokaryotic isolates of *C. hirsutus* and *C. pubescens* and in dikaryotic intraspecies pairings of these species. It was stated (Edwards, 1972; Edwards and Kennedy, 1973) that these coloured demarcation lines were of limited taxonomic value for those species. However, pigmented zones are not reported between isolates of the same species of *Hirschioporus* and such reactions have not been observed in this study. Furthermore, the similarity in the response of dikaryotic mycelia of *H. pargamenus* and *H. subchartaceus* to species of other genera confirms the close relationship of these species. *H. abietinus*, on the other hand, is characterized by being unable to resist the activity of other polypores in culture with it.

The mycelial interactions of the three *Hirschioporus* species display similar micro-morphology. Although mycoparasitism is not proved by the

observation of clasping branches without further morphological and physiological studies, the structure of these peculiar branches and their occurrence in the interaction zone strongly suggests a parasitic type of antagonism by *Hirschioporus* species. The success with which *H. pargamenus* is able to resist the overgrowth by other polypores is partly explained by the frequent observation of clasping branches. However, chemical inhibitions are also involved in these interactions. The gnarled, vesiculate hyphae and extrusions of cytoplasm are taken as evidence of the effects of chemicals diffusing into the agar and acting as toxins or substances disrupting membranes and upsetting the osmotic equilibrium in the hyphae. A similar proposal was advanced by Porter (1924) as a result of his studies of mycelial interactions in mixed cultures of the Fungi Imperfecti. I have found that yellowish diffusates in the interaction zones of confrontations involving *Trametes hispida*, *Gloeophyllum saepiarium* and *Fomes pini* are associated with yellow granules within and on the surface of the hyphae of these species. It is in these interactions with *Hirschioporus* species that extrusions of cytoplasm and empty hyphae not in contact with hyphae of the opposing species are observed. The lack of clasping branches in response to *Trametes hispida* when confronted by *H. abietinus*, for example, may be due to the death of hyphae of *Hirschioporus abietinus* exposed to toxic compounds diffusing in advance of the *T. hispida* hyphae. Further work with mycelia separated by dialysis membranes in culture would provide more information as to the significance of diffusible chemicals in these interactions. Such studies have been reported by Dennis and Webster (1971) for *Trichoderma viride* in relation to its production of non-volatile antibiotics which alter the morphology and eventually kill the

hyphae of species paired with it in culture.

The results which I have presented for the studies of coloured zones produced in mixed culture cannot be used to explain the formation of zone lines in the wood decayed by *Hirschioporus* species in the field. There is no indication of black "zone lines" similar to those seen in nature being formed in pure cultures of *Hirschioporus* species. Furthermore, there is no proof for the theory that "zone lines", observed in the wood decayed by *Hirschioporus* species, are produced by mycelial interactions. Extensive isolations of mycelium from each side of the line are required. Limited isolation from both sides of a black zone line in a log on which *H. abietinus* basidiocarps were collected, yielded mycelium identified as *Fomes pini*. It is clear that careful studies in nature are required to establish that the zone lines associated with a *Hirschioporus* species are actually produced by that organism. The usual practice of recognizing lines in the wood as belonging to a particular species based on the association with basidiocarps on the surface is not very precise.

Assuming that *Hirschioporus* species do interact with other fungi in nature, mycoparasitism and chemical inhibition could be significant phenomena in explaining the succession of fungi on decaying logs. Ecological studies concerning this aspect of wood-decay are generally lacking. Butcher (1968) and Shigo (1967) indicated that wood is primarily colonized by species of the Fungi Imperfecti and wood-staining ascomycetes in the initial stages of decay, and that basidiomycetes infected the wood later. The former have been shown to contribute little to the weight loss of the decaying wood compared to the activity of the basidiomycetes. Moreover, these initial colonizers are capable of removing free sugars and starch from the wood and in so doing inhibit the subsequent

colonization and decay by basidiomycetes (Toole, 1971; Hulme and Shields, 1970, 1972). Ascomycetes colonizing wood in culture also inhibit decay by basidiomycetes (Basham, 1966, 1973; Etheridge, 1957). Because inhibition of the growth of basidiomycete colonies was observed on malt agar, it is possible that the response may have been due to the action of antibiotics. Furthermore, the ability of *Hirschioporus* species to parasitize other fungi would give them a distinct advantage in the colonization and decay of previously infected wood. This was also the view held by Griffith and Barnett (1967) based on their report of the ability of 14 basidiomycetes including *H. pargamenus*, to parasitize the hyphae and spores of *Ceratocystis* spp. (blue-staining ascomycetes) in culture. In that same paper, they pointed out that parasitism of other fungi in wood is a source of nitrogenous compounds known to be scarce in this substrate.

Mycelial interactions in nature may also explain the localized distribution of *Hirschioporus* species on specific substrates. I have proposed this explanation based on my observation that in culture there is no substrate preference and based on the reports in the literature (Rhoads, 1918) that *P. pargamenus* and *P. abietinus* have been found side by side on the same log. However, I am not trying to explain substrate preferences on the basis of interactions alone, for it must be noted that we know very little of the specific requirements for temperature and moisture. These factors will no doubt vary with the habitat and physical structure of the substrate. Etheridge *et al.* (1972) have attributed the reduced and localized colonization of western hemlock by *Echinodontium tinctorum* to the observed antagonistic behaviour of "non-hymenomycetous fungi" growing in the exposed stubs. Further studies of the community

of organisms in wood decayed by *Hirschioporus* species in Western Canada may show that there are antagonists in certain substrates that are significant in explaining the puzzling host preferences.

TABLE 5. Production of coloured zones in culture

	Gloeophyllum saepiarium	*Fomes pini*	*Cerrena unicolor*	*Trametes hispida*	*H. abietinus*	*H. subchartaceus*	*H. pargamenus*
Hirschioporus pargamenus	wyl	brl	wyl	byl	wl	wl	0
Hirschioporus subchartaceus	wyl	brl	wl	byl	wl	0	
Hirschioporus abietinus	byl	brl	wl	wl	0		
Trametes hispida	brl	yl	byl	0			
Cerrena unicolor	brl	yl	0				
Fomes pini	brl	0					
Gloeophyllum saepiarium	0						

LEGEND:

0...no response

brl...dark yellow-brown zone

byl...dark blue zone in broader yellow zone

yl...broad yellow diffusion zone

wl...white, submerged line of demarcation

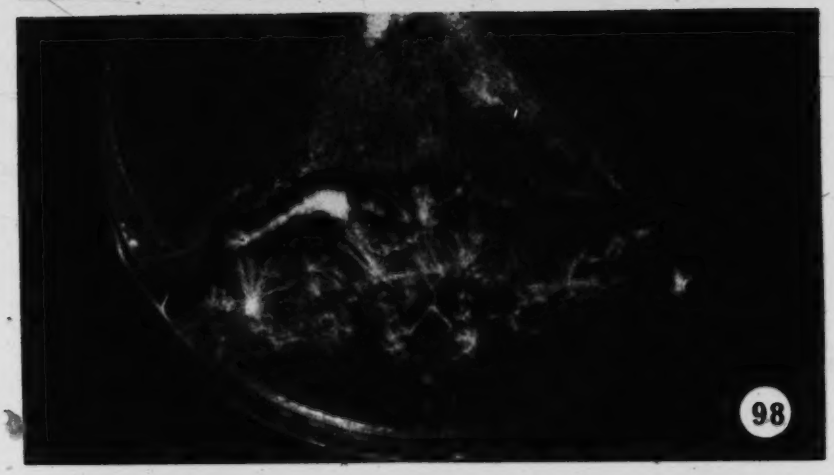
wyl...white demarcation line within a broader yellowish zone

FIGURE 97. Yellow interaction zone with narrower blue bands between colonies of *Trametes hispida* (T527) and *H. pargamenus* (T73). Reaction observed from the reverse after 3 weeks. X0.84

FIGURE 98. Surface of interaction zone between mycelia of *Trametes hispida* (top) and *H. pargamenus* (bottom). X1.2

FIGURE 99. Yellow-brown interaction zone (reverse) between colonies of *H. pargamenus* (T73) and *Fomes pini* (T540) after 3 weeks. X0.8

73
97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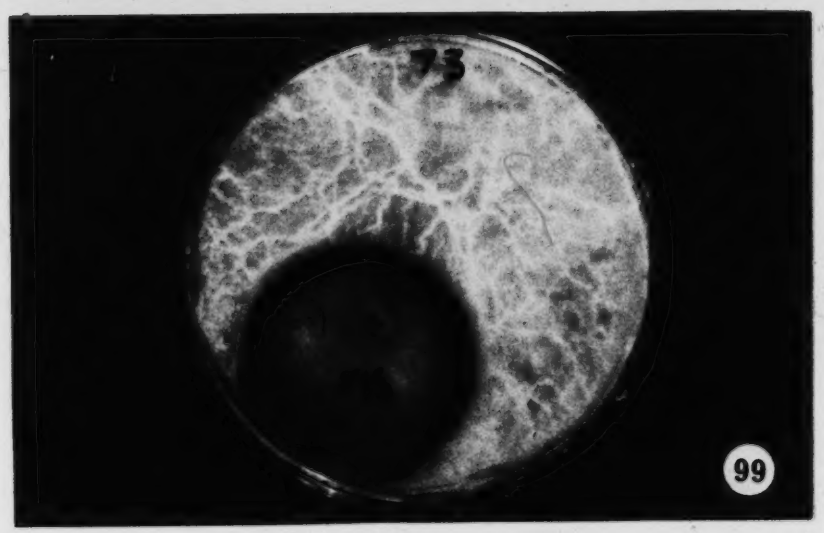

73
99

FIGURE 100. White demarcation line between paired dikaryotic isolates of *H. pargamenus*, T73 (left) and *H. subchartaceus*, T526 (right). X0.66

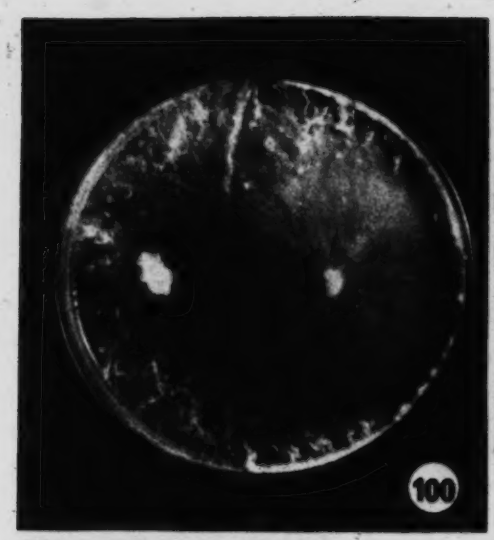
FIGURE 101, 102. Clasping branches in the interaction zone between isolates of *H. pargamenus*, T592 and *Fomes pini*. X1000.

FIGURE 103, 104. Extrusion of cytoplasmic material from the tips of *H. abietinus* (T532) hyphae confronted by mycelium of *Cerrena unicolor*. X1000.

FIGURE 105. Pairings of dikaryotic isolates of *H. pargamenus* (T73), *H. subchartaceus* (T526) and *H. abietinus* (T532) with mycelium of *Trametes hispida* (T527). A coloured zone occurs in confrontation with *H. pargamenus* mycelium. X0.4

FIGURE 106. Pairings of dikaryotic isolates of *Hirschioporus* species with *Fomes pini* (T540). Note that the same isolates of *Hirschioporus* are illustrated in Figures 105-107. Coloured zones are noted in confrontations with mycelia of all three *Hirschioporus* species. X0.4

FIGURE 107. Pairings of isolates of *Hirschioporus* species with *Cerrena unicolor*. Note the general absence of coloured zones. X0.4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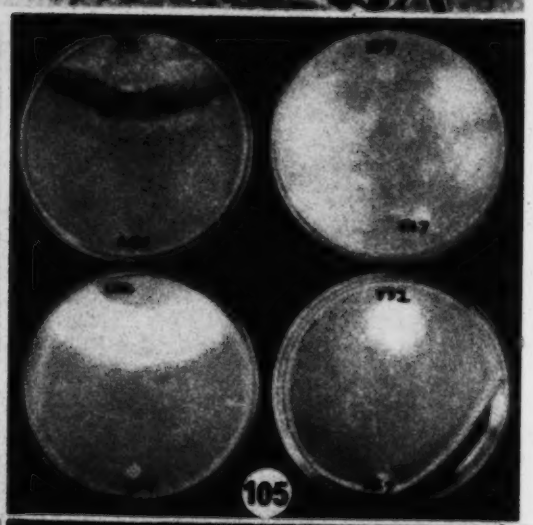
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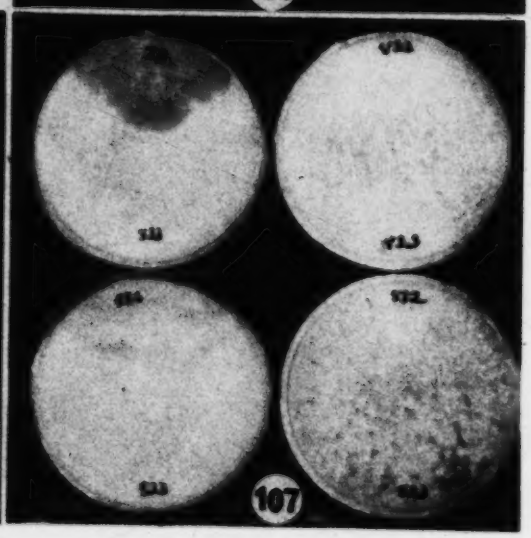
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CHAPTER VII

SUMMARY AND CONCLUSIONS

Classification of fungi in the Polyporaceae is in a confused state and at present, no system is universally accepted. Most of the difficulty in identification and classification of polypores arises from complete reliance upon a single taxonomic approach. Traditionally, species have been compared on the basis of macroscopic features of the reproductive phase of the life cycle. However, the macroscopic morphology of the basidiocarps of polypores produced in different environments is subject to variation which has resulted in unclear delineations of species. Fries' taxonomic studies of the Polyporaceae are based on macroscopic characteristics of basidiocarps and provide the foundation for all later systems of classification. Recognizing the artificiality of Fries' broad, heterogeneous genera, subsequent taxonomists have split off smaller groups of closely related taxa. Their resolution of species limits improved through the use of microscopic features of basidiocarps. But over-emphasis of such characters as hyphal systems has resulted in classifications (Cunningham, 1954, 1965) that are just as artificial and unsatisfactory for the identification of polypore species.

The three *Hirschioporus* species that form the nucleus of this study are common in North America and illustrate the problems extant in polypore taxonomy. Each taxon has been described as a species on the basis of basidiocarp morphology, but the delineation and recognition of the limits of these species based on their published descriptions has

presented difficulties. Their basidiocarps are highly variable in nature and as a consequence, mycologists have considered *H. subchartaceus* to be a form of *H. pargamenus*. In despair, others have found that the general preference of *H. pargamenus* for hardwoods and of *H. abietinus* for softwoods is the only distinguishing feature between these two taxa. The three species have been classified in the broad genus, *Polyporus*, and the somewhat narrower genus, *Coriolus*, but their inclusion in the small genus, *Hirschioporus*, emphasizes their close relationship.

Meaningful classification and identification of polypores hinges upon unambiguous species delimitations which can only be obtained by correlating information gained from studies of the complete life-cycle. Many diverse characters must be considered if correct taxonomic decisions are to be made and this involves the use of several approaches in the study of the organism. Once the limits of species are precisely determined, groupings into genera reflect better their interrelationship and provide the basis for a more natural classification. In this thesis, a natural classification is considered to be one in which the members of each group have many characters in common.

The various approaches that I have taken in the characterization of the three species, *H. pargamenus*, *H. abietinus* and *H. subchartaceus* have each contributed similarities and differences. The approaches are organized more or less chronologically in the order in which they have been used in the history of polypore taxonomy. Macro-morphology of basidiocarps has been followed by studies of their microstructure. But a more meaningful interpretation of basidiocarp micro-morphology has been attained by study of its development. The cultural approach to the study of these fungi emphasizes the growth and development of the

vegetative mycelium under standard conditions. Throughout this study the term vegetative mycelium has been used with reference to hyphae other than those which are part of the reproductive mycelium or the organized basidiocarp. In this report developmental studies were most important in correlating vegetative and reproductive phases of the life-cycle in nature and in culture. Genetical studies, almost dogmatically accepted by some mycologists as the ultimate criterion for species distinction, have evolved out of the cultural approach. However, examination of paired monokaryotic mycelia has shown certain anomalous results with *Hirschioporus* species which emphasize the need for supportive information. Genetical studies have prompted studies of the mycelial interactions of paired dikaryotic isolates as another source of taxonomic criteria for distinction of *Hirschioporus* species. When information from the several approaches is considered, species distinctions and close relationship in this genus become evident.

Basidiocarp Macrostructure

The close relationship between the three *Hirschioporus* species is illustrated by similarities in the macroscopic features of their basidiocarps. The most striking of these is the violaceous pore surface. But macro-morphology of the basidiocarp is a convenient source of features by which the three taxa can be identified. For example, the reddish, brittle context of basidiocarps of *H. abietinus* when dry can be used as a feature which distinguishes this species from *H. subchartaceus* and *H. pargamenus*. These two taxa have thicker, leathery to fibrous contexts which are concolorous with the basidiocarp surface. Generally,

H. subchartaceus basidiocarps can then be distinguished from those of *H. pargamenus* by virtue of the configuration of the hymenial surface which is soon tooth-like for *H. pargamenus* and poroid for *H. subchartaceus*. But hymenial configuration varies with the age of the basidiocarp and the environment in which it is produced. Consequently, intermediate forms are difficult to assign to either species without supportive information. The colour, zonation, and pubescence of the basidiocarp and its attachment must be regarded cautiously as distinguishing features. Their expression is often subject to variation with state of maturity of the basidiocarp and its exposure to differing environmental factors such as light, moisture, temperature and orientation with respect to the force of gravity during the course of its development. Precise description of characters such as colour, degree of pubescence and size requires examination of several populations in order to detect disjunctions in the range of variability for each species. Many of the less tangible tendencies towards distinction in such features as pubescence, are based on the relative abundance of various hyphal types. Distribution studies have revealed that the three taxa are widespread in Western Canada, and that the less studied species, *H. subchartaceus*, is frequently the cause of white-rot in *Populus* species. Since in Eastern North America more than one species may be found on a given substrate, the apparent preferences in Western Canada do not provide a conclusive distinction between the species.

Microstructure and Development of Basidiocarps in Nature

Mycologists attempting to improve upon the Friesian scheme of classification have utilized microscopic characters, mostly of elements in the hymenial layer. Since the anatomical studies of Corner (1932, 1953), analysis of hyphal types in basidiocarps has received increased attention from polypore taxonomists. In applying Corner's terminology to the hyphae in all basidiocarps, however, difficulty is encountered because of the simplicity of the original, functional concepts upon which identification of the various types are based. The origin and development of the different types of hyphae in the maturing basidiocarp must be known in order to be sure of their distinction.

In the basidiocarps of *Hirschioporus* species thin-walled, branched, multicellular, generative hyphae with clamp connections are the fundamental components that give rise to the other microscopic elements. In the growing portions of the basidiocarp, the long (1200-1500 μ), unbranched, skeletal cell is formed by the simultaneous growth and wall thickening of a terminal generative cell. In the context the multicellular, generative hyphae develop thickened walls in lateral branches and intercalary, non-growing cells. Corner's functional concept of hyphal types is inadequate for distinguishing skeletal cells from thick-walled generative cells because both components have a structural role. Basidia and capitate, crystal-incrusted cystidia composing the palisade-like hymenium are short, terminal cells of generative hyphae present in the tube walls. The larger spores of *H. subchartaceus* is the only distinctive micro-morphological feature. The developmental morphology of the microscopic, hyphal components of the basidiocarps is similar in

all three species. This observation supports the conclusion derived from studies of basidiocarp macrostructure, that they are closely related taxa.

Mycologists who have examined hyphal fragments from basidiocarps have not been successful in showing more meaningful relationships between polypore species. I contend that Corner's over-simplified, "mitic" concept of basidiocarp construction does not reflect sufficiently the variation in development and morphology for many polypores, and that the recognition of "hyphal systems" as he conceived them is inadequate for taxonomic purposes. The proper approach to the study of basidiocarp microstructure is to work out the pattern of development in terms of hyphal structure and organization. This developmental approach is strongly recommended as a source of information concerning interrelationships in the Polyporaceae.

Cultural Studies

Examination of the vegetative mycelium growing in standard cultural conditions is routine in the investigation of polypores. As in nature the generative hyphae and skeletal cells are similar for the three species. Moreover, their development in monokaryotic and dikaryotic mycelia is identical to that observed in the surface mats on logs and in natural basidiocarps. Thin-walled generative hyphae give rise to long (up to 1500 μ), terminal, thick-walled, unbranched skeletal cells and short, thin-walled branches with crystal-incrusted tips. In the older mycelium, generative cells become thick-walled as well. The similarity of these hyphae to the ones observed in natural basidiocarps of *Hirschioporus* species is the basis for my decision to use Corner's terminology to

describe the components of the vegetative mycelium and cultural basidiocarps rather than the terms used by Nobles in her key for the identification of cultures.

Macro-morphology of colonies is remarkably similar, but distinctions can be made between species on the basis of texture of the surface mats. For example, the aerial mycelium of *H. subchartaceus* is distinctly cottony, whereas that of *H. abietinus* is appressed and woolly and that of *H. pargamenus* is felted in tufts or reticulate strands. These textures are constant on artificial media and sterilized wood blocks.

Growth rate of colonies, if quantified more precisely as linear rate of linear extension, can be used to distinguish the three taxa, but care must be taken to consider the variability among several isolates of each species. Generally, *H. subchartaceus* colonies were observed to grow more rapidly. Although more studies are required to provide a complete spectrum of responses to various temperatures, comparative studies at 36°C and 18°C show that *H. abietinus* isolates do not tolerate higher temperature, while *H. subchartaceus* and *H. abietinus* isolates grow more rapidly than *H. pargamenus* at the lower temperature.

The development of basidiocarps of *Hirschioporus* species in culture is similar to the process in nature, although only the trama and hymenial layer can be recognized on cultural basidiocarps. As in nature distinctions in hymenial configuration and spore size are evident in the standard cultural environment.

Although limited distinctions between *Hirschioporus* species can be made on the basis of cultural studies, the major significance of this approach lies in the confirmation of relationships previously determined by morphological studies of basidiocarps. Moreover, studies of the

vegetative mycelium facilitate the recognition of various hyphal types that are difficult to discern in natural basidiocarps.

Genetical Studies

Proponents of the biological concept of the species advocate the use of genetical tests for conspecificity. However, the production of clamp connections when two monokaryotic isolates of the same species are paired is a single characteristic and one which merely indicates the initiation of the reproductive phase. It provides no guaranty that the mycelium will go on to produce fruiting bodies and viable spores. Moreover, such a criterion is useful only for those species bearing clamps on dikaryotic hyphae.

Macrae (1941) successfully applied this approach to distinguish *P. pargamenus* from *P. abietinus*, but she observed "hybridization" between a single isolate of *P. pargamenus* and isolates of the poroid *P. pargamenus* (*H. subchartaceus*). My studies of pairings between *H. pargamenus* and *H. subchartaceus* monokaryons revealed a general pattern of interspecies sterility. But likewise, results were not wholly negative because of "hybridization" observed between an isolate of *H. pargamenus*, T745, and isolates of *H. subchartaceus*. It must be emphasized that these hybrids were unstable and never developed fructifications. Anomalies in the complete interfertility of isolates of the same species took the form of unilateral dikaryotizations caused by common factor heterokaryons, mutations and loss in pairing ability in aged mycelia. However, the patterns of intraspecies fertility and interspecies sterility were confirmed by the results obtained by use of the Buller Phenomenon.

The results of genetical studies enabled me to draw boundaries between the species which corresponded to the delimitations set down by the results of morphological and developmental studies of the vegetative mycelium and basidiocarps. But in the case of *Hirschioporus*, taxonomic decisions based on the results of pairing studies alone are very difficult and certainly require supportive information. The interaction of monokaryotic mycelia resulted in white, submerged lines of demarcation that separated the colonies of different species. Within the interaction zone between monokaryotic mycelia of different species of *Hirschioporus*, peculiar clasping branches that resembled the absorptive structures on biotrophic mycoparasites were produced. The occurrence of these mycelial interactions supports the conclusion that mycelia of the different species are not compatible.

Mycelial Interactions in Mixed Culture

The interaction of mycelia of dikaryotic isolates of the different *Hirschioporus* species paired in culture also resulted in the formation of white lines of demarcation and clasping branches. When paired with dikaryotic isolates of species of other genera commonly associated with them in nature, distinctive interactions were observed. The differences in the response of *Hirschioporus* species provide further support for their distinction. The macroscopic expression of mycelial reactions can take the form of coloured zones between the colonies. For example, pairing isolates of *H. pargamenus* and *H. subchartaceus* with an isolate of *Trametes hispida* resulted in the formation of a yellow zone with a narrower blue band, but when isolates of *H. abietinus* were paired with

the *Trametes hispida* isolate no coloured zone was observed. The reaction, however, was largely a response of the mycelium of *Trametes hispida* because pairing this isolate with an isolate of *Cerrena unicolor* resulted also in the production of a similar yellow zone and blue band. Therefore, these interactions must be used comparatively in the distinguishing of *Hirschioporus* species. Microscopically, the interaction consisted of chemical inhibition causing death or morphological aberrations in the hyphae and of apparent parasitism whereby hyphae of *Hirschioporus* isolates clasped foreign hyphae by means of swollen, sickle-shaped branches. Further morphological and physiological studies are required to confirm the conclusion that these branches are an indication of parasitism by *Hirschioporus* species.

Interactions of mycelia of different species is discussed with respect to the explanation for zone lines and succession of fungi in decayed wood in nature. For the most part, extensive studies in this area of fungal ecology are lacking and conclusions cannot be drawn. I have proposed that the distribution of different *Hirschioporus* species in nature on separate substrates may be related to the particular mycoflora and mycelial interactions with invading *Hirschioporus* species. But, mycelial interactions in the field environment await future investigation.

Smith (1968) has stated that "our system of classifying the higher basidiomycetes will never approach even an attainable degree of 'naturalness' unless we give proper emphasis to all the features of the species". I believe that the many characters required for these taxonomic studies must be obtained by using several approaches. Differences in macroscopic basidiocarp features will probably remain the most practical

features for identification, but their delineations must be supported by other information. Similarities, on the other hand, in several features of all phases of the life-cycle are of paramount importance in the recognition of interrelationships between species, genera and families. For example, Overholts (1953) reported that basidiocarps of *Polyporus versatilis* resembled those of *P. pargamenus* and *P. abietinus* with respect to pubescence, colour of pore surface and form of cystidia in the hymenium. Cultural studies of this species by Bakshi *et al.* (1969) showed similarities in the vegetative mycelium (cystidia) to that of *P. pargamenus* and *P. abietinus*, while Bakshi and Chouhdury (1961) demonstrated that isolates of *P. versatilis* were intersterile when paired with isolates of the latter two species. Further developmental studies will probably indicate that *P. versatilis* should be included in the genus, *Hirschioporus* whose key features are the violaceous pore surface containing crystal-incrusted cystidia and cystidia-like branches in cultured mycelia.

The following summary lists the practical distinctions of the natural basidiocarps of the three species in Western Canada:

H. pargamenus. Hymenial surface poroid but soon conspicuously tooth-like; spores 5-7.4x1.8-2.2 µ; context white, fibrous when dry, ≥1 mm thick; white-rot of *Betula* sp.

H. subchartaceus. Hymenial surface distinctly poroid; spores 6-11x 2-2.5 µ; context white, fibrous when dry, ≥1 mm thick; white-rot of *Populus* sp.

H. abietinus. Hymenial surface poroid; spores 5.5-7.4x2-2.7 µ; context reddish, brittle when dry, ≤1 mm thick; white-rot of conifers.

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APPENDIX I

FRESH MATERIAL

Collections of *Hirschioporus pargamenus*

Collection No.	Location*	Date	Substrate
T30	Winterburn	05.13.71	*Betula*
T73	New Norway	07.26.71	"
T360	Winterburn	08.24.71	"
T592	Edmonton	10.04.72	"
T597	Winterburn	04.13.73	"
T728	What Cheer, Iowa	09.01.72	*Prunus*
T743	Ann Arbor, Mich.	11.12.72	*Betula*
T745	Ann Arbor, Mich.	11.11.72	*Quercus*

Collections of *Hirschioporus abietinus*

T16	Wabamun Lake	09.17.70	*Picea*
T17	Mt. Robson, B.C.	09.18.70	"
T18	Mt. Robson, B.C.	09.18.70	"
T21	Edmonton	09.29.70	"
T34	Yoho Nat. Park, B.C.	05.20.71	*Pinus*
T48	Little Smoky	06.29.71	*Picea*
T49	Edson	07.05.71	"
T52	Edson	07.05.71	"
T54	Edson	07.05.71	"
T56	Hoadley	07.11.71	"
T58	Wolf Creek	07.05.71	"
T61	Robb	07.05.71	"
T84	Cypress Hills, Sask.	07.27.71	*Pinus*
T87	Cypress Hills, Sask.	07.27.71	"
T93	Cypress Hills, Sask.	07.27.71	"
T126	Telfordville	08.04.71	*Picea*
T127	Telfordville	08.04.71	"

*Alberta except where noted.

Collection No.	Location	Date	Substrate
T130	Telfordville	08.04.71	*Picea*
T150	Drayton Valley	08.04.71	"
T170	Elk Is. Nat. Park	08.06.71	"
T172	Elk Is. Nat. Park	08.06.71	"
T181	Swan Hills	08.10.71	"
T184	Swan Hills	08.10.71	*Pinus*
T185	Whitecourt	08.10.71	*Picea*
T190	Whitecourt	08.10.71	"
T191	Whitecourt	08.10.71	"
T197	Wabamun Lake	08.12.71	"
T206	Wabamun Lake	08.12.71	"
T243	Entwistle	08.16.71	"
T244	Entwistle	08.16.71	"
T259	Wolf Creek	08.16.71	"
T262	Wolf Creek	08.16.71	"
T266	Wolf Creek	08.16.71	"
T269	Robb	08.17.71	"
T276	Fickle Lake	08.17.71	"
T280	Fickle Lake	08.17.71	"
T288	Fickle Lake	08.17.71	"
T292	Fickle Lake	08.17.71	"
T293	Fickle Lake	08.17.71	"
T298	Fickle Lake	08.17.71	"
T302	Coalspur (F.T.Rd.)	08.17.71	*Pinus*
T306	Fairfax L. (F.T.Rd.)	08.17.71	*Picea*
T308	Fairfax L. (F.T.Rd.)	08.17.71	"
T311	Hazeau Bridge (F.T.Rd.)	08.18.71	*Pinus*
T313	Fish Lake (Nordegg)	08.18.71	*Picea*
T314	Fish Lake (Nordegg)	08.18.71	"
T317	Fish Lake (Nordegg)	08.18.71	"
T319	Fish Lake (Nordegg)	08.18.71	"
T321	North Ram R., (F.T.Rd.)	08.18.71	"
T323	North Ram R., (F.T.Rd.)	08.18.71	"
T327	Ram River Falls (F.T.Rd.)	08.18.71	*Pinus*

Collection No.	Location	Date	Substrate
T332	Clearwater Bridge (F.T.Rd.)	08.18.71	*Pinus*
T336	James River (F.T.Rd.)	08.19.71	"
T337	James River (F.T.Rd.)	08.19.71	*Picea*
T341	James River (F.T.Rd.)	08.19.71	*Pinus*
T342	Sundre	08.19.71	*Picea*
T354	Winterburn	08.24.71	"
T364	Winterburn	08.24.71	"
T365	Spruce Grove	08.24.71	"
T367	Spruce Grove	08.24.71	"
T371	Spruce Grove	08.24.71	"
T373	Wabamun Lake	08.24.71	"
T376	Winterburn	08.24.71	"
T377	Winterburn	08.24.71	"
T378	Dawson Creek, B.C.	08.27.71	"
T400	Dawson Creek, B.C.	08.27.71	"
T412	Wonowon, B.C.	08.27.71	"
T419	Wonowon, B.C.	08.27.71	"
T422	20 mi. N. Wonowon, B.C.	08.28.71	"
T423	40 mi. N. Wonowon, B.C.	08.28.71	"
T424	40 mi. N. Wonowon, B.C.	08.28.71	"
T435	Prophet River, B.C.	08.28.71	"
T437	Prophet River, B.C.	08.28.71	*Pinus*
T438	Prophet River, B.C.	08.28.71	"
T444	Ft. Nelson, B.C.	08.29.71	*Picea*
T454	30 mi. S. Wonowon, B.C.	08.30.71	"
T459	Boundary Lakes	08.30.71	*Pinus*
T460	Boundary Lakes	08.30.71	"
T462	Clear Hills	08.30.71	*Picea*
T478	Grimshaw	08.30.71	"
T488	Hotchkiss	08.30.71	"
T492	Keg River	08.31.71	"
T493	Keg River	08.31.71	"
T507	Lesser Slave Lake	08.30.71	"
T508	Lesser Slave Lake	08.30.71	"

Collection No.	Location	Date	Substrate
T510	Lesser Slave Lake	08.30.71	*Picea*
T539	Twin Lakes	09.26.71	"
T543	Twin Lakes	09.26.71	"
T558	Lake Eden	10.02.71	"
T560	Entwistle	09.26.71	"
T585	Warburg	05.24.72	"
T587	60 mi. S. Grande Prairie	07.08.72	"
T594	Hay River, N.W.T.	10.05.72	"
T611	Waterton	07.28.72	"
T706	Itasca Lake, Minn.	08.25.72	*Pinus*
T627	Jasper Nat. Park	07.22.73	*Picea*
T638	Fox Creek	07.12.73	*Abies*
Collections of *Hirschioporus subchartaceus*			
T13	Wabamun Lake	09.17.70	*Populus*
T14	Wabamun Lake	09.17.70	"
T22	Edmonton	09.29.70	"
T23	Spruce Grove	10.02.70	"
T24	Spruce Grove	10.02.70	"
T26	Spruce Grove	05.13.71	"
T31	Spruce Grove	05.12.71	"
T33	Bow Valley Prov. Park	05.21.71	"
T35	Wabamun Lake	06.10.71	"
T36	Wabamun Lake	06.10.71	"
T37	Wabamun Lake	06.10.71	"
T50	Wolf Creek	07.05.71	"
T55	Lower Mann Lakes	06.30.71	"
T65	Bittern Lake	07.26.71	"
T67	Bittern Lake	07.26.71	"
T68	Bittern Lake	07.26.71	"
T72	New Norway	07.26.71	"
T91	Cypress Hills, Sask.	07.27.71	"
T100	Edgely, Sask.	07.29.71	"
T102	Edgely, Sask.	07.29.71	"

Collection No.	Location	Date	Substrate
T105	Edgely, Sask.	07.29.71	*Populus*
T112	Lestock, Sask.	07.29.71	"
T114	Lestock, Sask.	07.29.71	"
T120	Lanigan, Sask.	07.29.71	"
T122	Borden, Sask.	07.29.71	"
T123	Borden, Sask.	07.29.71	"
T129	Warburg	08.04.71	"
T133	Warburg	08.04.71	"
T153	Drayton Valley	08.04.71	"
T155	Drayton Valley	08.04.71	"
T159	Drayton Valley	08.04.71	"
T160	Moon Lake	08.04.71	*Populus*
T162	Moon Lake	08.04.71	"
T171	Elk Is. Nat. Park	08.06.71	"
T173	Swan Hills	08.10.71	"
T177	Swan Hills	08.10.71	"
T188	Swan Hills	08.10.71	"
T193	Wabamun Lake	08.12.71	"
T195	Wabamun Lake	08.12.71	"
T200	Wabamun Lake	08.12.71	"
T207	Cooking Lake	08.14.71	"
T221	Forestburg	08.14.71	"
T223	Forestburg	08.14.71	"
T224	Big Knife Prov. Park	08.14.71	"
T227	Gooseberry Lake	08.15.71	"
T233	Innisfree	08.15.71	"
T235	Innisfree	08.15.71	"
T247	Entwistle	08.16.71	"
T249	Entwistle	08.16.71	"
T251	Carrot Creek	08.16.71	"
T257	Carrot Creek	08.16.71	"
T263	Wolf Creek	08.16.71	"
T294	Fickle Lake	08.17.71	"
T295	Fickle Lake	08.17.71	"

Collection No.	Location	Date	Substrate
T338	James River (F.T.Rd.)	08.19.71	*Populus*
T346	Caroline	08.19.71	"
T348	Caroline	08.19.71	"
T353	Carrot Creek	08.16.71	"
T355	Winterburn	08.24.71	"
T356	Winterburn	08.24.71	"
T358	Winterburn	08.24.71	"
T361	Winterburn	08.24.71	"
T368	Spruce Grove	08.24.71	"
T380	Whitecourt	08.26.71	"
T384	Grande Prairie	08.27.71	"
T386	Demmitt	08.27.71	"
T387	Demmitt	08.27.71	"
T404	Fort St. John, B.C.	08.27.71	"
T406	Fort St. John, B.C.	08.27.71	"
T426	Trutch, B.C.	08.28.71	"
T436	Prophet River, B.C.	08.28.71	"
T439	Prophet River, B.C.	08.28.71	"
T442	Kledo River, B.C.	08.29.71	"
T443	Kledo River, B.C.	08.29.71	"
T445	Ft. Nelson, B.C.	08.29.71	"
T446	Ft. Nelson, B.C.	08.29.71	"
T463	Clear Hills	08.30.71	"
T465	Clear Hills	08.30.71	"
T469	Worsley	08.30.71	"
T472	Grimshaw	08.30.71	"
T473	Grimshaw	08.30.71	"
T474	Grimshaw	08.30.71	"
T479	Deadwood	08.30.71	"
T481	Deadwood	08.30.71	"
T483	Hotchkiss	08.31.71	"
T485	Hotchkiss	08.31.71	"
T489	Hotchkiss	08.31.71	"
T494	Peace River	08.31.71	"

Collection No.	Location	Date	Substrate
T495	Peace River	08.31.71	*Populus*
T501	Lesser Slave Lake	08.30.71	"
T503	Lesser Slave Lake	08.30.71	"
T506	Lesser Slave Lake	08.30.71	"
T513	Prince Albert, Sask.	08.28.71	"
T516	Edmonton	09.20.71	"
T526	Entwistle	09.26.71	"
T530	Entwistle	09.26.71	"
T541	Twin Lakes	09.26.71	"
T542	Twin Lakes	09.26.71	"
T549	Magnolia	09.03.71	"
T556	Lake Eden	10.02.71	"
T562	Lake Eden	10.02.71	"
T588	60 mi. S. Grande Prairie	07.08.72	"
T589	60 mi. S. Grande Prairie	07.08.72	"
T590	Wabamun Lake	07.01.72	"
T591	Moose Jaw, Sask.	07.--.70	"
T593	Hay River, N.W.T.	10.05.72	"
T595	Carson Lake	10.21.72	"
T596	Westlock	10.--.72	"
T599	Surprise Lake	06.22.73	"
T602	Whitecourt	07.12.73	"
T628	Surprise Lake	07.21.73	"
T630	Surprise Lake	07.21.73	"
T635	Genesee	09.16.73	"
T636	Genesee	09.16.73	"
T705	Itasca Lake, Minn.	08.25.72	"
T710	Itasca Lake, Minn.	08.25.72	"
T713	Itasca Lake, Minn.	08.25.72	"
T742	Ann Arbor, Mich.	11.11.72	*Betula*
T744	Ann Arbor, Mich.	11.12.72	*Prunus*

APPENDIX II

HERBARIUM MATERIAL

Accession No.	Location*	Substrate	Identified as
Collections of *Hirschioporus pargamenus*			
ALTA-2793	Calmar	*Betula*	*P. pargamenus*
ALTA-2794	Calmar	*Betula*	"
ALTA-2801	Economy Lake, N.S.	(hardwood)	"
ALTA-2805	Brookside, N.S.	*Fagus*	"
ALTA-2812	Edmonton	*Betula*	"
ALTA-2815	Lake Eden	*Betula*	"
ALTA-2821	Purgatory, Mass.	(hardwood)	"
ALTA-2822	Purgatory, Mass.	*Quercus*	"
ALTA-2823	Winchester, Mass.	*Quercus*	"
ALTA-2824	Princeport, N.S.	(wood)	"
ALTA-2825	Hubbardston, Mass.	*Quercus*	"
ALTA-3697	Pinawa, Man.	*Betula*	"
ALTA-4001	Edmonton	*Betula*	"
ALTA-4887	Elk Island	*Betula*	"
ALTA-5043	Winterburn	*Betula*	"
DAOM-30472	Petawawa, Ont.	*Betula*	*H. pargamenus*
DAOM-9452	Halfway House, N.B.	*Betula*	"
Lowe 3724	Great Smoky Mts., Tenn.	*Vitis*	*P. pargamenus*
Lowe 1931	Syracuse, N.Y.	*Acer*	"
Collections of *Hirschioporus abietinus*			
ALTA-2607	Robb	*Picea*	*P. abietinus*
ALTA-2608	Robb	*Picea*	"

*Alberta except where noted

Accession No.	Location	Substrate	Identified as
ALTA-2609	Jasper	*Picea*	*P. abietinus*
ALTA-2615	Edmonton	*Picea*	"
ALTA-2616	Whitecourt	*Picea*	"
ALTA-2614	Waterton	*Abies*	"
ALTA-2620	Whitecourt	*Pinus*	"
ALTA-2621	Vancouver, B.C.	*Thuja*	"
ALTA-2622	Carrot Creek	*Pinus*	"
ALTA-2623	Wolf Creek	*Pinus*	"
ALTA-2624	Wolf Creek	*Pinus*	"
ALTA-2628	Hartland, N.B.	*Picea*	"
ALTA-2629	Edmonton	*Picea*	"
ALTA-2630	Edmonton	*Picea*	"
ALTA-2632	Winterburn	*Larix*	"
ALTA-2635	Edmonton	*Picea*	"
ALTA-2803	Whitecourt	*Picea*	"
ALTA-3714	Whitecourt	*Picea*	"
ALTA-4353	Kerava, Finland	*Picea*	"
ALTA-4372	Gorge Creek	*Picea*	"
ALTA-4049	Jasper	*Picea*	*P. pargamenus*
ALTA-2805	Edmonton	*Picea*	*P. pargamenus*
ALTA-5010	Mitsue Lake	*Picea*	*P. abietinus*
DAOM-72305	Kelly's camp, Gaspé, P.Q.	*Picea*	*H. abietinus*
DAOM-73846	Luss, Loch Lomond, Scot.	*Pinus*	"

Collections of *Hirschioporus subchartaceus*

ALTA-2611	Spruce Grove	*Populus*	*P. abietinus*
ALTA-2792	Gregoire Lake	*Populus*	*P. pargamenus*
ALTA-2795	Jasper	*Populus*	"
ALTA-2796	Long Lake	*Populus*	"
ALTA-2797	Entwistle	*Populus*	"
ALTA-2798	Entwistle	*Populus*	"
ALTA-2799	Entwistle	*Populus*	"
ALTA-2800	Carrot Creek	*Populus*	"

Accession No.	Location	Substrate	Identified as
ALTA-2802	Edmonton	*Populus*	*P. abietinus*
ALTA-2804	Crimson Lake	*Populus*	*P. pargamenus*
ALTA-2806	Rocky Mt. House	*Populus*	"
ALTA-2807	Whitecourt	*Populus*	"
ALTA-2808	Gorge Creek	*Populus*	"
ALTA-2809	Gorge Creek	*Populus*	"
ALTA-2810	Edmonton	*Populus*	"
ALTA-2811	Edmonton	*Populus*	"
ALTA-2810	Edmonton	*Populus*	"
ALTA-2814	MacKenzie Hwy.	*Populus*	"
ALTA-2813	Widewater	*Populus*	"
ALTA-2816	Edmonton	*Populus*	"
ALTA-2817	Faust	*Populus*	"
ALTA-2818	Faust	*Populus*	"
ALTA-2819	Faust	*Populus*	"
ALTA-4002	Edmonton	*Populus*	"
ALTA-4003	Donnelly	*Populus*	"
ALTA-4004	Huntsville, Ont.	*Populus*	"
ALTA-4374	Long Lake	*Populus*	"
ALTA-5000	Lacombe	*Populus*	"
DAOM-17876	Hudson Bay, Sask.	*Populus*	*H. pargamenus*
DAOM-5216	Constance Bay, Ont.	*Populus*	"
fragment isotype	La Plata Mts., Col.	*Populus*	*C. subchartaceus*
RLG-219	Big Creek, Mont.	*Populus*	*P. subchartaceus*
Lowe 6291	Routt Nat. For., Col.	*Populus*	*P. pargamenus*
RLG-3431	Enterprise, N.W.T.	*Populus*	"
RLG-3591	Whitecourt	*Populus*	"
Lowe 6771	Uncompahgre For., Col.	*Populus*	*P. subchartaceus*

APPENDIX III

MEDIA USED IN CULTURAL STUDIES .

I. Nobles' Malt Agar (Nobles, 1965)

malt extract (Difco)	12.5 g
bacto-agar (Difco)	20.0 g
distilled water	1000 ml

II. 3% Malt Extract Agar

malt agar (dehydrated)	45.0 g
distilled water	1000 ml

III. Gallic Acid Agar (Nobles, 1965)

(a) malt extract	15.0 g
bacto-agar	20.0 g
distilled water	900 ml
(b) gallic acid	5.0 g
distilled water	100 ml

(a) and (b) were autoclaved separately and mixed after cooling slightly.

IV. 2% Malt Agar plus Glucose and Bactopeptone (Larcade, 1970)

malt extract	20.0 g
glucose	20.0 g
bactopeptone	1.0 g
bactoagar	20.0 g
distilled water	1000 ml

V. Aspen Sawdust Agar and Pine Sawdust Agar (a modified Czapek-Dox medium) (Collins and Lyne, 1970)

$NaNO_3$	1.0 g
$(NH_4)_2C_4H_4O_6$ (ammonium tartrate)	0.5 g
K_2HPO_4	1.0 g

$MgSO_4 \cdot 7H_2O$	0.5 g
$FeSO_4 \cdot 7H_2O$	0.01 g
KCl	0.5 g
bactoagar	20.0 g
glucose	5.0 g
pine or aspen sawdust	20.0 g
distilled water	1000 ml

VI. Faro's Agar (Faro, 1972)

$(NH_4)_2C_4H_4O_6$	1.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	2.88 mg
$FeSO_4 \cdot 7H_2O$	2.78 mg
$CuSO_4 \cdot 5H_2O$	0.74 mg
$MnSO_4 \cdot 4H_2O$	0.67 mg
$ZnSO_4 \cdot 7H_2O$	2.88 mg
thiamine-HCl*	120 µg
glucose	30.0 g
bacto-agar	15.0 g
distilled water	1000 ml

*Thiamine-HCl was prepared as a stock solution, filter-sterilized and added to the slightly cooled autoclaved medium.